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# IN VIVO SISTER CHROMATID EXCHANGES: A TEST SYSTEM MONITORING ENVIRONMENTAL GENOTOXICITY.

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# IN VIVO SISTER CHROMATID EXCHANGES: A TEST SYSTEM MONITORING ENVIRONMENTAL GENOTOXICITY

by

Bichitra nanda Nayak

A Dissertation
submitted to the
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ABSTRACT

IN VIVO SISTER CHROMATID EXCHANGES: A TEST SYSTEM MONITORING ENVIRONMENTAL GENOTOXICITY

An in vivo sister chromatid exchange (SCE) assay system for monitoring environmental genotoxicity, using bone marrow cells has been developed, tested and applied to groups of laboratory and outdoor maintained inbred mice, as well as laboratory maintained and freshly caught wild mice from various geographic locations in southwestern Ontario. The best results were obtained with nine, serial, intraperitoneal injections of 5-bromo-2'-deoxyuridine (BUdR) (40 ug/g of body weight) and 5-fluorodeoxyuridine (FUdR) (2 ug/g of b.w.) and then with 5 ug/g of b.w. of colchicine, given on the 27th hour following the first injections of BUdR/FUdR. The mice were sacrificed 3 hours later.

Chromosomal aberrations such as chromatid breakages, gaps, fragments, radial figures, metacentric chromosome, chromosome with twisted chromatid, centromeric chromatin filaments and Y chromosome breakage, particularly in pro-metaphases, occurred more frequently with the earlier time schedule (13-17 hr) than in the 27-30 hr time schedule. Increasing the dose of BUdR produced no substantial increase in aberrations, however, FUdR at higher dose levels increased chromatid aberrations. X and Y chromosomes showed asynchronous chromatid differentiation

compared to the rest of the metaphase chromosomes.

Using the 27-30 hr injection schedule, the control male mice of C3H/HeJ and C57BL/6J maintained in the laboratory gave a mean ± SEM baseline SCE value of 3.42 ±0.07 and 3.62 ±0.08 respectively. The female mice of the same strains gave 5.00 ±0.03 and 5.71 ±0.08 SCE/cell respectively. Males obtained from natural populations in southwestern Ontario had a higher mean SCE value (6.02 ± 0.16) as did the inbred males maintained in outdoor enclosures (5.08 ± 0.22). The response of male mice appeared higher compared with female mice under similar conditions. Wild mice maintained in the laboratory for a period of six months or over, gave SCE values similar to those of the control mice (3.46 ± 0.12). The SCE values in wild caught mice and in enclosure maintained inbreds were inversely proportional (r=-0.49) to the distance between the sites where the animals were either collected or maintained and the nearest major industrial centres.

Based on the differences in SCE values between laboratory maintained mice and wild mice as well as laboratory maintained and inbred mice housed at various outdoor sites, together with the geographic patterns in the SCE levels, it is suggested that the in vivo SCE analysis in bone marrow cells using the bromodeoxyuridine substitution method, has the potential of being an early warning surveillance system for the general levels of environmental genotoxic agents.

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## ABBREVIATIONS

| | |
|---|---|
| AAF | acetylaminofluorene |
| BUdR | 5-bromo-2'-deoxyuridine |
| b.w. | body weight |
| Cd | cadmium |
| CHO | Chinese hamster ovary |
| CP | cyclophosphamide |
| dc | deoxycytidine |
| DNA | deoxyribonucleic acid |
| DEN | diethyl nitrosamine |
| DMN | dimethyl nitrosamine |
| EMS | ethyl methanesulfonate |
| FUdR | 5-fluorodeoxyuridine |
| HBSS | Hanks balanced salt solution |
| M.I. | mitotic index |
| MMS | methyl methanesulfonate |
| MMC | mitomycin-C |
| MNNG | N-methyl-N'-nitro-N'-nitroso-guanidine |
| MNT | micronucleus test |
| PCB | polychlorinated biphenyl |
| RNA | ribonucleic acid |
| SCD | sister chromatid differentiation |
| SCE | sister chromatid exchange |

# CHAPTER I

## GENERAL INTRODUCTION

Environmental pollutants pose a serious problem to man, his genome and his ecosystem. These pollutants are increasing dramatically from various sources, such as industrial and domestic effluents, combustion products of fossil fuels, agricultural chemicals and other man-made compounds found in food, water, air, soil and vegetation. A variety of chemical pesticides, food toxins and other agriculturally used chemicals have been found in significant quantity in human blood, adipose tissues, milk, and other body fluids (Haugh, 1982a).

Damage to DNA by environmental pollutants has been and will continue to be a major cause of cancer (Doll et al., 1970; Weisberger, 1976; Doll, 1977; Hiatt et al., 1977; Blot et al., 1977; Wynder and Gori, 1977; Chrisp and Fisher, 1980; Lederberg, 1981; Pallitti et al., 1982) and genetic defects (Beckman and Nordström, 1976; Benditt, 1977; Ames, 1979). Moreover, these substances may also contribute to such ailments as heart disease (Benditt, 1977), aging (Burnet, 1974), and cataracts (Jose, 1979).

Considering the seriousness of pollution and the introduction of new, hazardous chemicals such as polychlorinated biphenyls (PCBs), vinyl chloride, into the human environment and the ecosystem, there is considerable

need for developing monitoring systems capable of measuring the level of the DNA damaging agents in the environment thereby avoiding the necessity of identifying specific chemicals.

During the past few years, a number of tests have been developed to detect mutagenicity and carcinogenicity. These include:

1) The Micronucleus Test (MNT): This testing procedure examines the acentric fragments of chromosomes generally in polychromatic erythrocytes but also in other interphase cells. This test serves as a rapid screening method for chromosome breaking (clastogenic) agents and agents which interfere with normal mitotic cell division (Heddle, 1973; Jenssen et al., 1974; Schmid, 1975; Maier and Schmid, 1976).
2) The Conventional Karyotypic Analyses: These analyses are done using either lymphocytes from humans exposed to various clastogenic agents or somatic and germinal cells of whole animals and assessing various types of numerical and structural chromosomal aberrations (Evans and Scott, 1969; Evans and O'Riordan, 1975; Raposa 1978).

The above two tests have limited application because high doses of the test compound are required and cell deaths are frequently encountered.

3) The Dominant Lethal Test: It gives a measure of the genetic effects through increased mortality of the embryos as a consequence of lethal mutations induced in germ cells (Bateman and Epstein, 1971). This test may give false negative results because some mutagenic compounds do not reach the germinal cells (Anderson, 1979). Also, certain agents like vinyl chloride which is a known carcinogen (Maltoni and Lafemine, 1974), mutagen (Bartsch and Montesano, 1975) and clastogen (Purchase et al., 1976) produce a negative result in dominant lethal assays in mice (Anderson, 1979) because they require metabolic activation (Green and Hathaway, 1975).

4) The Heritable Translocation Test: This is a genetic-cytogenetic test in which progeny of treated mice are examined for sterility and reduced fertility. Males showing such reproductive problems are examined for translocations in germ cells. The difficulties with this test include: i) the rate of production of heritable translocations is dependent on germ cell stages (Russell and Matter, 1980) and, ii) only alkylating agents are clearly shown to induce heritable translocations (Generoso et al., 1978).

5) Specific Locus Testing: This measures the rate of point mutations involving recessive alleles at seven loci in house mice (Russell, 1951). Although this

test is of particular value in assessing radiation hazards to man (Russell and Matter, 1980) its value in assessing chemical mutagens has not been fully explored (Malling and Valcovi, 1977).

6) The Host Mediated Assay: This assay system was developed by Gabridge and Legator (1969) and employs a microbial indicator which is injected into the peritoneal cavity of a mammal. This test is not a true mammalian cell system unless mammalian cells are used as indicators. With this testing procedure, the host is responsible for absorption, distribution, metabolism, detoxification and excretion of the compounds. Any genetic variability in the hosts used will affect the final test results. The serious limitations of this test are: cell survival in the host, effects of selection pressures on heterogenous cell populations, and the spontaneous mutation rates.

7) The Ames Salmonella/Microsomal Test: It is essentially an in vitro screening method for detecting bacterial mutagens. This test was developed by Ames and his co-workers (1975) using specially constructed Salmonella typhimurium auxotrophic for histidine. This test is used with or without a microsomal activation system. Other organisms such as Escherichia coli (Bridges, 1972) and yeast (Parry, 1972; Zimmerman, 1971, 1975) have also been used. Limitations of this test include an inability to detect benzene,

stilbestrol and most metallic mutagens (Hollstein and McCann, 1979) and the fact that the activating enzymes (Ames et al., 1973) may not produce metabolites in vitro identical to the metabolic array produced in vivo (Bigger et al., 1978).

8) The Somatic Cell in Tissue Culture: This provides a system to study the mutagenic effects of many chemical agents and the molecular and biochemical pathways involved in mutations. This is an in vitro procedure and requires metabolic activation (Anderson, 1979).

9) The Mammalian Cell Transformation: This test is based on the ability of mammalian cells transformed to form colonies in soft agar (Purchase et al., 1976). The relationship between transformation and mutation is still not well understood. The culturing conditions if not controlled properly, will affect the final results.

10) In Vivo Somatic Mutation Test (Mouse Spot Test): This test was originally described by Russell and Major (1957). The test consists of treating mouse embryos that are heterozygous at a number of specific coat colour loci with the test chemicals in utero. A few weeks later the young are examined for mosaic patches of brown, tan or gray fur against the background of normal black fur. The test is devised to provide a relatively quick prescreening for the

more extensive tests of heritable genetic damage. The major disadvantages of the test include: i) spots can also be caused by melanocyte insufficiency (Russell and Matter, 1980) and ii) certain compounds or metabolites do not cross the placental barrier and thus may not reach the target cells in the embryos (Russell and Matter, 1980).

11) Sister Chromatid Exchange (SCE) Analysis: Sister Chromatid Exchange (SCE) is a cytogenetic procedure for the detection of cellular DNA damage. SCE involves symmetrical interchange between homologous (sister) chromatids in a replicating chromosome without apparent alteration in chromosome morphology (Perry and Evans, 1975). This assay system has been proven a sensitive, rapid, and quantitative measure of DNA damage (Perry and Evans, 1975).

Most of the above tests are not good candidates for the general monitoring of environmental genotoxic agents. Among those that appear suitable are: the micronucleus test, the mammalian spot test, the dominant lethal test and the sister chromatid exchange analysis. Since none of the tests have been fully explored for monitoring the environment, the present study is concerned with doing this for the SCE test.

The SCE test was selected because induction of SCE can be accomplished with a relatively low dose of the test compound (Marquardt and Bayer, 1977) as compared to other tests such as the dominant lethal (Ehling, 1975) micro-

nucleus (Matter and Grauwiler, 1974), heritable translocation (Lang and Adler, 1977) and the mouse spot test Fahrig, 1975). The *in vivo* SCE testing procedure utilizes the whole animal and so does not require an activating component. The *in vivo* system also provides a means of studying synergistic or inhibiting effects of many physical and chemical agents. For instance, Watanabe *et al.*, (1982) have shown an interaction between cadmium (Cd) and polychlorinated biphenyls (PCBs) in causing mutations in mice, Tice *et al.*, (1980) have shown that phenobarbital alone had no effect on the induction of SCE in DBA mice but showed a synergistic effect with benzene. Similarly, Rao *et al.*, (1979) have reported that benzo[a]pyrene may act as a co-mutagen for benzo[e]pyrene and 2-acetylaminofluorene (2-AAF).

Therefore, the purpose of the present study was to examine in detail the suitability of the *in vivo* sister chromatid exchange assay, utilizing the bone marrow cells of house mouse, *Mus musculus domesticus*, for general monitoring of environmental genotoxic agents.

House mice were selected because: a) they have 20 pairs of easily identifiable acrocentric chromosomes, b) there are a number of well established inbred strains of mice available for use as controls, c) the animals are easily bred and handled and are readily maintained in laboratory and non-laboratory conditions, and finally d) wild mice are easily collected.

The bone marrow cells were chosen as the test cells for SCE analysis because these are a highly proliferative group of cells and can easily be collected and assayed.

The other objectives of the study were to: a) evaluate the effects of various experimental conditions on sister chromatid differentiation and sister chromatid exchanges b) assess the type of chromosomal aberrations that were expected due to BUdR, FUdR, colchicine and MMC, and c) establish the baseline SCE values in selected control inbred strains of mice and in mice under different environmental conditions.

# CHAPTER II

# DEVELOPMENT OF SCE ANALYSIS SUITABLE FOR WILD MICE

## 1. INTRODUCTION

### A. Historical Development of the Technique

Taylor et al., (1957) first demonstrated the occurrence of sister chromatid exchange (SCE) through autoradiography of $^{3}H$-labelled thymidine in plant chromosomes. Zakarov and Egolina (1972) showed that when the chromosomes of Chinese hamster ovary cells were treated with bromodeoxyuridine (BUdR) for two rounds of replication and subsequently stained with Giemsa, the sister chromatids stained differentially. The unifilarly substituted chromatids, that is BUdR substitution in one of the two polynucleotide DNA double helices, stained dark and the bifilarly substituted (BUdR incorporated in both strands) stained light. This procedure enabled the visualization of SCEs under a light microscope without the use of radioisotopes and autoradiography. Latt (1973) showed a similar staining pattern in metaphase chromosomes from human lymphocytes when treated with a fluorochrome (Hoechst 33258) and examined through a UV microscope.

A further technical development was made by Perry and Wolff (1974) and Kato (1974a). These workers showed that BUdR treated chromosomes when stained with acridine orange and Giemsa produced 'Harlequin' chromosomes. Korenberg and Freedlender (1974) introduced a heat pretreatment with

Giemsa staining for the induction of differential staining without any additional treatment with fluorochromes such as Hoechst 33258. This method had the advantage of producing permanent cytological preparations that could be viewed with a light microscope.

The first demonstration that a mutagenic chemical could be detected by the BUdR-SCE technique was by Latt (1974a) who found a high frequency of SCEs in cultured human lymphocytes treated with mitomycin-c (MMC). Since then, numerous *in vitro* SCE studies have been reported (Perry and Evans, 1975; Evans *et al.*, 1977; Solomon and Bobrow, 1975; Natarajan *et al.*, 1976; Abe and Sasaki, 1977a, 1977b; Takehisa and Wolff, 1977; Wolff and Takehisa, 1977; German *et al.*, 1977; Crossen *et al.*, 1978; Beek and Obe, 1975). BUdR labelling *in vivo* was first demonstrated by Bloom and Hsu (1975) who observed SCEs in chick cells labelled *in ovo*. The first mammalian *in vivo* methods were described independently by Vogel and Bauknecht (1976) in mouse bone marrow and by Allen and Latt (1976a) in mouse spermatogonial cells.

Whether BUdR is injected as a single large dose or multiple small doses over a period of DNA synthesis (S-phase), BUdR causes tissue toxicity affecting the number of cells and the mitotic index. Deoxycytidine, a deoxyribonucleoside, has been used to counteract BUdR toxicity (Allen and Latt, 1976b).

Another compound, 5-fluorodeoxyuridine (FUdR), a halogenated pyrimidine which blocks DNA synthesis

by inhibiting thymidylate synthetase (Hartman and Heidelberger, 1961; Heidelberger, 1965; Santi et al., 1974) has been injected intraperitoneally along with injections of BUdR in in vivo studies (Vogel and Bauknecht, 1976; Bauknecht et al., 1977) in order to increase the BUdR incorporation into DNA. However, FUdR itself is not incorporated into DNA (Choudhuri et al., 1958).

With the BUdR substitution in vivo, one of the major difficulties has been the rapid depletion of the injected BUdR due to dehalogenation in the liver (Barrett and West, 1956). After one hour, only 20% of the intraperitoneally injected BUdR concentration is left in the serum (Schweiger, 1972). Attempts to overcome this problem have included: multiple intraperitoneal injections of BUdR (Allen and Latt, 1976a,b; Vogel and Bauknecht, 1976), continuous subcutaneous injections (Perra and Mattias, 1976), intravenous injections (Schneider et al., 1977), subcutaneous implantation of BUdR tablets (Allen et al., 1977) and the injection of BUdR adsorbed to activated charcoal (Russev and Tsanav, 1973; Ramirez, 1980). All modes of administration have been found satisfactory with the exception of the latter because it has been reported that the mutagens themselves may be adsorbed to the activated charcoal (Ramirez, 1980)

B. SCE as a Phenomenon

The molecular mechanism and the mutational basis of SCE have not been fully understood. SCE formation appears to be tightly coupled with DNA synthesis (Kato, 1974b).

Hypotheses to explain the formation of SCEs have included: errors in DNA replication (Kato, 1974c; Latt, 1981) and breakdown in post replication repair processes (Kato, 1973; Bender et al., 1974; Wolff et al., 1974; Kato, 1977b). Recently, Painter (1980) in his replicon model for the formation of SCEs has suggested that the DNA regions near the junction of replicon clusters are uniquely susceptible to double stranded breaks during replication. If the rejoining of the broken strands is delayed, one would expect an increased chance of SCE formation at this junction (Natarajan et al., 1981). Carrano et al., (1978) have shown that increasing doses of certain alkylating agents produced a parallel increase in SCEs and mutations. Each mutagen showed a characteristic ratio of SCE to point mutation. Swenson et al., (1980) have reported that certain SCE inducing alkylating agents cause $O^6$-alkylation of guanine thereby creating critical lesions leading to mutation (Pegg, 1977; Singer, 1979). More work is necessary to understand the detailed aspects of molecular and cellular processes involved in the formation of SCE and its relationship to point mutations and chromosomal aberrations.

C. SCE in the Measurement of Environmental Genotoxicity

A large number of mutagens/carcinogens have been found to induce SCE. Table 1 shows a list of mutagenic agents and their in vivo SCE inducibilities in mammalian cells.

Table 1: Effect of Certain Environmental Agents on In Vivo SCE Induction in Mammalian Cells

| Agents | Effect | References |
|---|---|---|
| A. DNA synthesis (S-phase) Independent | | |
| I. Ionizing radiation | | |
| X-rays | ± (weak) | Gatti and Olivieri (1973); Perry and Evans (1975); Evans (1977). |
| γ-rays | ± (weak) | Solomon and Bobrow (1975). |
| II. Antibiotics | | |
| bleomycin | ± (weak) | Evans (1977); Giebhart and Kappauf (1978). |
| B. DNA Synthesis (S-phase) Dependent | | |
| I. Non-ionizing radiation | | |
| u.v light | + | Perry and Evans (1975) Kato (1973); Wolff et al., (1974) |
| II. Labelled radio-isotopes: 3H-thymidine | + | Gibson and Prescott (1978) |
| III. Alkylating agents | | |
| cyclophosphamide(CP) | + | Stetka and Wolff (1976); Schmid (1976); Allen and Latt (1976a); Roszinsky Köcher and Rohrborn (1979). |
| methyl methanesulfonate (MMS) | + | Stetka and Wolff (1976); Marquardt and Bayer (1977) |
| ethyl methanesulfonate (EMS) | + | Stetka and Wolff (1976) |
| nitrogen mustard | + | Schmid (1976) |
| quinacrine mustard | + | Schmid (1976) |
| thiotepa | + | Stetka et. al., (1977); |
| triaziquone | + | Vogel and Bauknecht (1976) |
| triethylene melamine | + | Yamamoto and Kikuchi (1981) |
| IV. Polycyclic Aromatic Hydrocarbons | | |
| benzo(a)pyrene | + | Rozinsky-Kocher et. al.(1979) |
| benzanthracene | + | " |
| benzo(b)fluoranthrene | + | " |
| benzo(e)pyrene | + | " |

Table 1 (contd)

| Agents | Effect | References |
|---|---|---|
| phenanthrene | + | Rozinsky-Kocher et.al. (1979) |
| chrysene | - | " |
| dibenzanthracene | + | " |
| styrene | + | DeRaat (1978) |
| 3,4 benzo(a)pyrene | + | Bayer and Bauknecht (1977) |
| 7,12 dimethylbenzanthracene | + | " |
| benzene | + | Tice et.al. (1980) |
| urethane | + | Csukas et. al. (1979) |
| V. Cytostatic Antibodies | | |
| mitomycin-C | + | Kram et. al. (1979); Kram et. al. (1981); Yamamoto and Kikuchi (1981); Kato and Shimada (1975); Lin and Alfi (1975); Allen and Latt (1976a); Isnii and Bender (1978). |
| adriamycin | + | Perry and Evans (1975) |
| daunomycin | + | Schmid (1976b) |
| VI. N-nitroso Compounds | | |
| methyl nitrosoguanidine | ±(weak) | Wolff et. al. (1977) |
| dimethyl nitrosamine | + | Latt (1979) |
| diethyl nitrosamine | - | Latt (1979) |
| VII. Antimetabolites (DNA and Protein Syuthesis Inhibitors | | |
| mercaptopurine | + | Schmid (1976b) |
| methotrexate | - | " |
| fluorouracil | + | " |
| VIII. Fungal Toxins | | |
| aflatoxins | + | Takehisa and Wolff (1977) |
| IX. Miscellaneous | | |
| viruses | + | Nichols et. al. (1978); Brown and Crossen (1976); Kato and Sandberg (1979); Kurvink et. al. (1978). |
| 5-ethoxy caffeine | + | Perry and Evans (1975) |
| 2-acetylamino fluorene | + | Latt (1979) |
| 2-aminofluorene dimethyl-1-phenyltriazene | + | Latt (1979) |

The objectives of this section were:

i) to develop an *in vivo* SCE detection system using bone marrow cells of both inbred and wild *Mus musculus domesticus* capable of measuring environmental genotoxicity and to evaluate factors which may affect SCE levels;

ii) to establish the base levels of SCEs in selected laboratory maintained inbred strains of mice; and

iii) to test the sensitivity of the system with mitomycin-C, a known mutagen and SCE inducer.

## 2. MATERIALS AND METHODS

### A. Materials

Animals: C3H/HeJ, C57BL/6J, DBA/2J (Jackson Laboratory, Bar Harbor, Maine, U.S.A.) and $F_1$ mice from matings of C3H males and C57BL females were used in the present study as control animals.

The animals were maintained in the laboratory in 80 x 25 x 20 cm stainless steel cages with heat treated wood chips as bedding in rooms normally at 25°C. They were fed rodent laboratory chow (Purina Co.) and tap water *ad lib*. The animals were given 14 hours of light and 10 hours of darkness.

Cells: The hemopoietic tissues of femur and tibia were analyzed.

Chemicals: 5-bromo-2'-deoxyuridine (BUdR), 5-fluorodeoxyuridine (FUdR), deoxycytidine, mitomycin-C (MMC) (Sigma Co. St. Louis, U.S.A.), colchicine (ICN Nutritional Biochemicals, Cleveland, Ohio, U.S.A.), 0.075M KCl and RPMI 1640 medium (GIBCO, Burlington, Ontario), absolute methanol and glacial acetic acid (Fisher Co., Toronto, Ontario) and several chromosome stains such as Giemsa stains from Fisher Co., GIBCO, Merck (W. Germany) and basic fuchsin (British Drug Houses Ltd.) were used.

Solutions

i) Hanks Balanced Salt Solution (HBSS): HBSS was used as the solvent for BUdR, FUdR and deoxycytidine. The solution was made as per the formula given in Appendix I. The solution was sterilized at 106°C and 117 Kilopascals for 15 min. The pH after sterilization was 7.0.

ii) BUdR, FUdR, and Deoxycytidine Solutions: Appropriate quantities of BUdR, FUdR and deoxycytidine were separately dissolved in sterile HBSS. Each of the above solutions was filter sterilized using 0.22 um pore size filter, (Millipore Co.)

Colchicine Solution: The required quantity of colchicine was similarly weighed and dissolved in sterile 0.95% NaCl. The solution was then filter sterilized.

0.075M KCl was obtained from GIBCO, Canada and was used without dilution as a flushing medium and hypotonic solution for marrow cells.

Fixative Solution: The bone marrow cells were fixed in a solution of 3 parts of absolute methanol and 1 part glacial acetic acid.

Mitomycin-C Solution: MMC was dissolved in either sterile 0.95% Saline or sterile Dulbecco's phosphate buffer saline. The formula for Dulbecco's phosphate buffer is given in Appendix I. The solution was autoclave-sterilized before use. All solutions were made 24 hr before use. The tubes were wrapped in aluminum foil to protect them from light. BUdR, FUdR, deoxycytidine and MMC solutions were stored at -10°C while the colchicine solution was stored at 4°C.

iii) Staining Solutions: Four types of Giemsa stains, Gurr (London, England), Merck (West Germany), GIBCO (Canada) and Fisher (Canada) and also other stains such as aceto-orcein and basic fuchsin (Feulgen Staining) were tried.

iv) Photographic Solutions: The photographic solutions were prepared and used according to the instructions of the manufacturer with slight modifications.

B. General Description of the Procedure

BUdR, FUdR and deoxycytidine solutions were injected intraperitoneally and separately under reduced light using a one-ml disposable plastic syringe and a 26G needle. BUdR, FUdR and in some cases, deoxycytidine, were given as serial injections either at 30, 45, or 60 min intervals. The last dose of FUdR was injected at twice the normal dose. Colchicine was given as a single injection, a few hours before the

animals were sacrificed.

Specific Modifcations

Chemicals

i) Colchicine: Three different doses of colchicine (5 ug/g, 10 ug/g and 20 ug/g of b.w.) were tried using male C3H mice. Doses were given intraperitoneally one half hr before sacrificing. The animals received no other treatments. This was done in order to determine which dose level produced enough metaphases without any chromosomal contraction. In all other experiments, 5 ug/g of b.w. of colchicine was injected at different times for 2,3 and 4 hr periods following the serial injections of BUdR, FUdR and deoxycytidine.

ii) BUdR: BUdR at concentrations of 30, 40, 50 and 60 ug/g of b.w. with 2 ug/g of b.w. of FUdR and 1 ug/g of b.w. of deoxycytidine were evaluated.

iii) FUdR: FUdR at concentrations of 0,2,4,8,10,20,30 and 40 ug/g of b.w. with 40 ug/g of b.w. of BUdR with and without deoxycytidine have been tested.

iv) Deoxycytidine: 1 ug/g of b.w. of deoxycytidine was tried along with various doses of BUdR and 2 ug/g of b.w. of FUdR.

v) Flushing Medium: Three types of flusing medium for bone marrow cells were tried. These included phosphate buffer saline, RPMI 1640 medium and 0.075M KCl.

vi) Fixative: Two fixatives were tried; one composed of 3 parts absolute methanol and 1 part glacial acetic acid, and the other consisted of 3 parts absolute ethanol and one part glacial acetic acid.

Injection Schedules

These schedules refer to the number of serial injections (either 30, 45 or 60 min intervals) and the times when the animals received the colchicine and were sacrificed. The number of hourly injections which were evaluated with various combinations of BUdR, FUdR and deoxycytidine were 8,9,10, and 11. Also twelve injections at 45 min intervals and 18 injections at 30 min intervals were tried. For the 13-17 hr time schedule, the animals received colchicine on the 13th hr following the first injections of BUdR/FUdR and were sacrificed on the 17th hour. Other schedules that were tested included 13-15, 13-17, 17-21, 21-24, 24-27, 27-30 hr where the first figure in the time schedule refers to the number of hours following the first injection of BUdR/FUdR when colchicine was injected and the second figure is the time when the animals were sacrificed.

C. Method of Collection and Preparation of Bone Marrow Cells

The animals were sacrificed by cervical dislocation. The skin was removed from the hind limb using curved forceps. The muscle was stripped away leaving the femur and tibia exposed. The proximal and distal ends of the femur were cut in order to open the marrow cavity. The tibia was also

cut at its proximal end and about one third from its distal end. Using 0.5-0.8 ml of warm (37°C) 0.075M KCl and a 1-ml disposable syringe with a 26G needle, the bone marrow cells were flushed 2 or 3 times into a graduated centrifuge tube containing about 3 ml of 0.075M KCl prewarmed to 37°C. The tube was incubated for 18-20 min at 37°C.

The cells were then suspended with a nine-inch pasteur pipette and centrifuged for 8 min at 200 x g using a bench centrifuge. The supernatant was removed. Then, about 1-1.5 ml of freshly prepared cold fixative (3 parts methanol and 1 part glacial acetic acid) were added to the cell pellet. The fixative was quickly (15-30 seconds) removed using a pasteur pipette and 2 ml of fresh, cold fixative were added. The tubes were then sealed with parafilm and refrigerated for 20 min at 5-8°C. The tubes were then centrifuged for 5 min at 200 x g. The supernatant was then taken off using a pasteur pipette and 2-3 ml of fresh fixative were added slowly along the wall of the tube while the cells were gently resuspended. The above steps were repeated for additional 15, 10 and 10 min periods of refrigeration. After final centrifugation the tubes were sealed with parafilm and refrigerated for 10-12 hr at 5-8°C. The tubes were centrifuged at 200 x g for 5 min. The supernatant was taken off with a pasteur pipette leaving a thin layer of fixative over the cell pellet. About 0.5 to 0.75 ml of fresh fixative were added and the cells were then

resuspended. Using a nine inch pasteur pipette, 2-3 small drops of cell suspension were dropped on a cleaned, chilled slide from a 2-4 inch height. After the drops began to spread and the edge of the drops began contracting, the cell surface was blown to hasten drying. This was repeated several times. Then the slides were warmed by quickly passing them through the flame of an alcohol lamp. Care was taken not to overheat the slide.

Between 3-5 slides were made from each culture tube. The slides were stored at room temperature for at least 24 hr before they were differentially stained. All slides were stained within 48 hr after they were made. Slides that were stored for a longer period of time produced poorly differentiated metaphases.

D. Staining Procedure

The differential staining was performed as suggested by Korenberg and Freedlender (1974) with slight modifications. The chromosome slides were warmed for 15-25 min at 60°C then heated in 1M phosphate buffer (1M $Na_2HPO_4$ and 1M $NaH_2PO_4 \cdot H_2O$) (pH 8.1) for 15 min at 89°C. Then they were rinsed briefly in the above buffer at room temperature and stained in 5% Fisher Giemsa solution made in Gurr's buffer (pH 6.8) using tablets (Gurr Co.) for 8 min. After that, they were washed in a solution made with 2 ml of the above phosphate buffer (pH 8.1) and 48 ml of distilled water. The slides were air dried and stored for 1-2 weeks before examination.

Other Giemsa staining solutions from Gurr Co., Merck Co. and GIBCO were also tried.

E. Microscopic Examination and Photomicroscopy

The slides were examined with a 100 x oil immersion objective lens. About 800-1000 cells were counted in estimating the mitotic index. One hundred metaphases were used for determining the percentage of differentiated metaphases. Between 20 and 25 well spread differentiated metaphases with good morphology were examined for SCEs under oil immersion (100 x) objective lens.

Selected metaphases were photographed using either Kodak high contrast copy film or Kodak techinal pan film. These metaphases were photographed under a 100 x oil immersion objective using a combination of special blue-green and blue filters (Hoya, Japan).

Kodak technical pan film 2415 (Ester AH Base) and Kodak high contrast film were used. The latter gave better resolution and definition. The technical pan film was developed in D-19 at 20°C for 4 min with continuous agitation, rinsed in Kodak stop bath for 15 seconds, then fixed in Kodak rapid fixer at 20°C for 5 min with continuous agitation. Finally, the film was washed in running water at 20°C for 20 min. Kodak high contrast copy film was developed in D-19 at 20°C for 5½ min and the rest of the processing was the same as for the technical pan film.

Various combinations of printing paper and exposure

conditions were tried. Medium and high contrast papers were found to be suitable. The prints were developed in Dektol at 20°C for one min, then rinsed in tap water and fixed.

## 3. RESULTS AND DISCUSSION

### A. General Results

Ten and 20 ug/g of b.w. of colchicine injected half an hour and one hour before sacrificing the mice produced highly contracted chromosomes. A dose of 5 ug/g of b.w. of colchicine given 3 and 4 hr before sacrificing the mice on the other hand gave a sufficient number of metaphase chromosomes of good morphology. For animals weighing less than 16 g, 4 ug/g of body weight of colchicine gave the best result. Since most of the animals employed in the present study weighed between 16 and 25 g of b.w., 5 ug/g of b.w. of colchicine were normally used. The 13th hr following the first injection of BUdR/FUdR was chosen as the time when colchicine was added since the *in vivo* cell cycle of bone marrow cells in Chinese hamster cells was 9.5 hr (Fremuth *et al.*, 1976). According to these workers, the cell cycle phases in the Chinese hamster include: the pre-DNA synthetic period $G_1$ 1.9 hr, the DNA synthetic period (S-phase) 6.0 hr, post DNA synthetic period ($G_2$) 0.7 hr and mitosis (M) 0.9 hr. In the absence of specific information on murine bone marrow cell cycle parameters, the duration of cell cycle phases in

hamsters was used in planning some of the experiments. Figure 1 shows a light microscopic view of bone marrow preparations with different types of interphase cells and a metaphase plate. Figure 2 shows a typical spread of murine bone marrow metaphase using the described procedure. Figures 3 and 4 show differentiated metaphase plates in inbred and wild mice with 5 and 8 SCEs respectively. These metaphase plates also illustrate that the sizes of exchanges may also vary.

B. Effects of Injection Schedules on Mitotic Indices and Percentage of Differentiated Metaphases

Table 2 shows the mitotic indices, percentage of differentiated metaphases and SCEs for two injection schedules, 13-15 hr and 13-17 hr in cells collected from the femur of C57BL and C3H males. A comparison between the two injection schedules; 13-15 hr and 13-17 hr, revealed significant differences ($p < .01$) in mitotic indices and percentage of differentiated metaphases. The 13-15 hr injection schedule not only produced a lower percentage of differentiated metaphases, but also showed a greater number of incompletely differentiated metaphases. These results suggested that the 13-17 hr schedule was preferred.

C. Effect of Various Concentrations of BUdR on Mitotic Indices, Percentage of Differentiated Metaphase and SCEs

BUdR at doses of 30,40 50 and 60 ug/g of b.w. with 2 ug/g of b.w. of FUdR and 1 ug/g of b.w. of deoxycytidine was

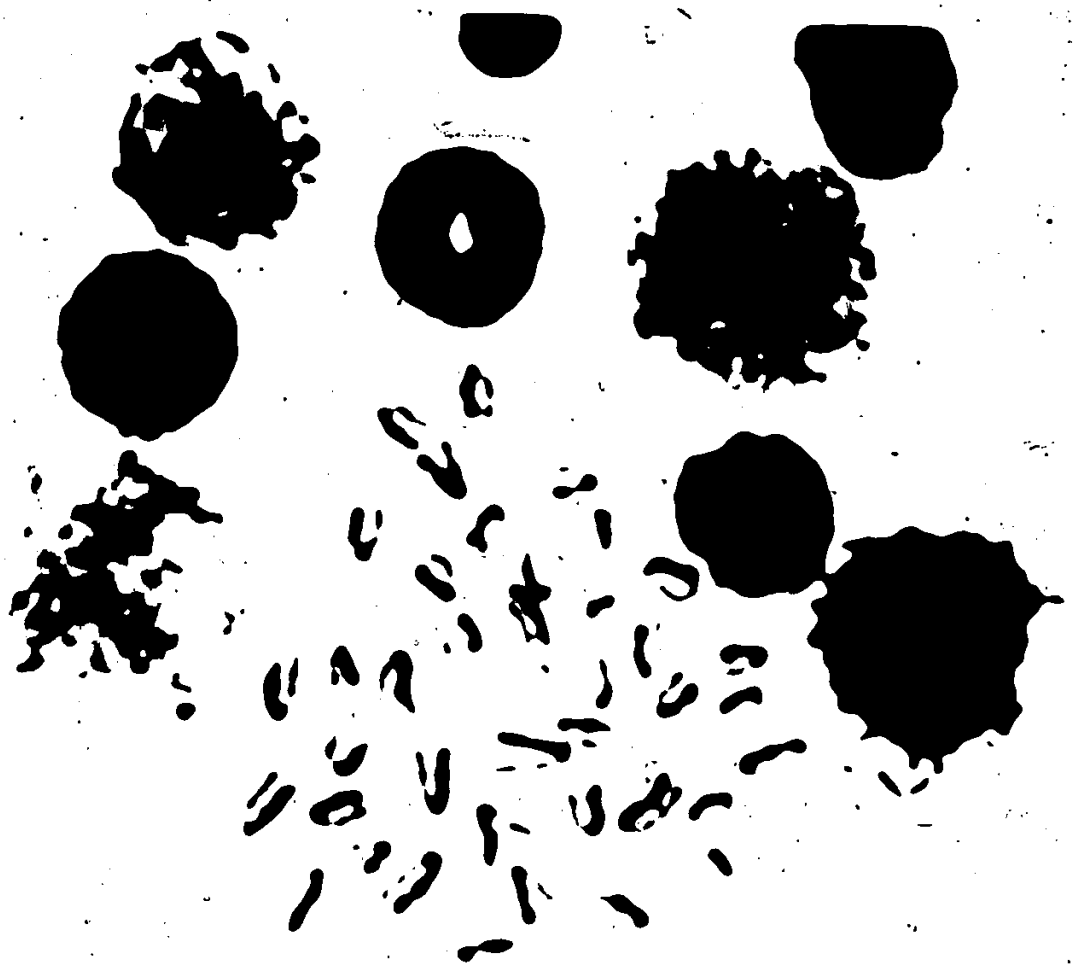

Fig. 1. A light microscopic view of bone marrow preparations (x6,000; Giemsa stain).

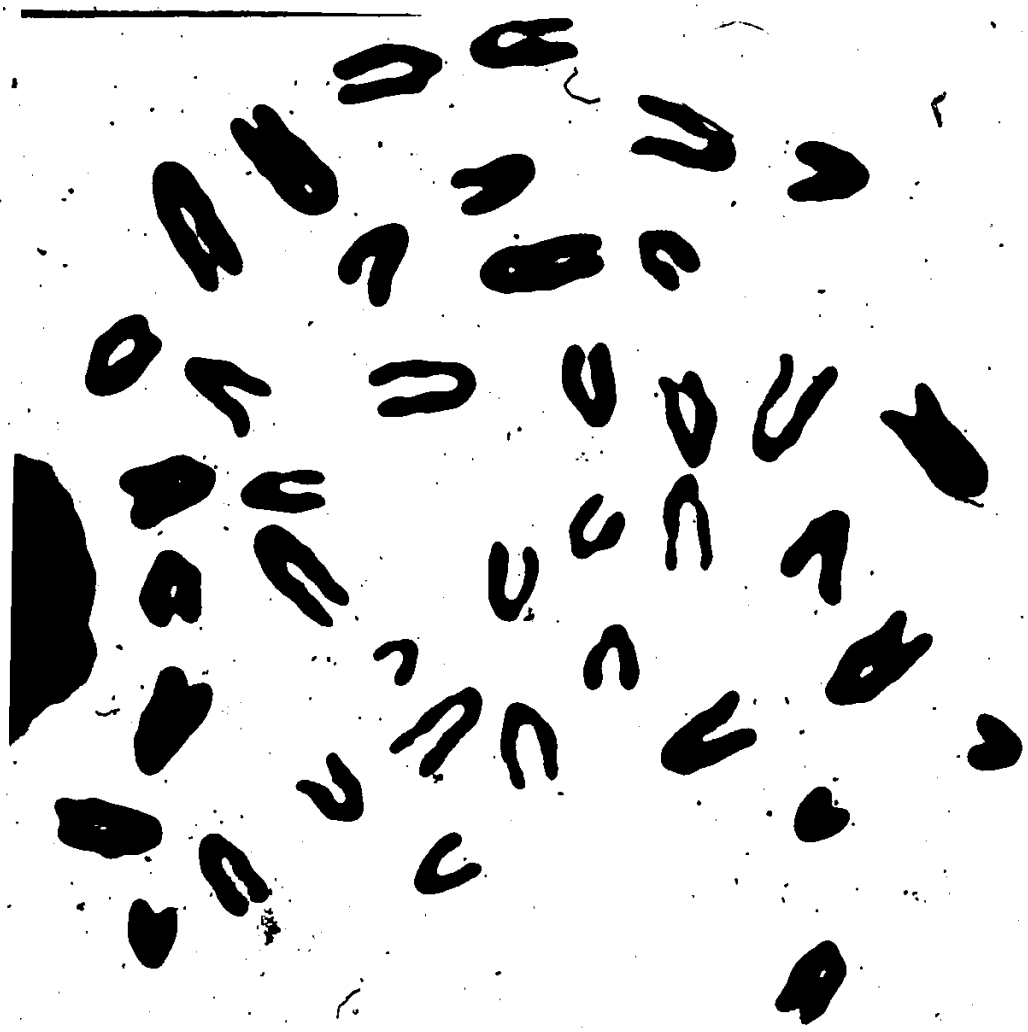

Fig. 2. A typical murine bone marrow metaphase spread (x5,000; Giemsa stain).

Fig. 3. Differentiated metaphases with terminal and centromeric SCEs in inbred mouse (x4,000; Giemsa stain).

Fig. 4. Differentiated metaphaes with terminal, interstitial and centromeric SCEs in wild mouse. Exchanges are small but detectable. (x5,400; Giemsa stain)

Table 2: Mitotic Indices, Percentage of Differentiated Metaphases and SCEs in C57Bl and C3H Males With 13-15 and 13-17 hr Injection Schedules.

| Injection Schedule* | No. and Strain of Animals | | Mitotic Index | Percentage of Differentiated Metaphases | Average SCE/Cell |
|---|---|---|---|---|---|
| 13-15 hr | 4 | C57BL | 3.80 | 16.00 | 1.45 |
| 13-15 hr | 4 | C3H | 3.75 | 14.00 | 1.34 |
| 13-17 hr | 2 | C57BL | 6.70 | 30.11 | 2.04 |
| 13-17 hr | 2 | C3H | 7.80 | 24.45 | 1.90 |

*The 13 hr refers to the time when colchicine was injected following the first injection of BUdR/FUdR/deoxycytidine. The second time refers to the hour when the animals were sacrificed following 9 hourly injections of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and bihourly injections of 1 ug/g of b.w. of deoxycytidine with 5 ug/g of b.w. of colchicine at the 13 hr.

tried to determine the dose effects on cell viability, percentage of differentiated metaphases and SCE levels. The animals received 9 hourly injections of BUdR, FUdR and bihourly injections of deoxycytidine with 5 ug/g of b.w. of colchicine on 13th hr for four hr. The results are presented in Table 3.

The results indicate that the higher doses of BUdR had an adverse effect on the mitotic index. At 60 ug/g of b.w. of BUdR, tissue toxicity was seen resulting in reduced mitotic indices and poorly differentiated metaphases. At 30 ug/g of b.w. the percentage of differentiation was low and chromatid differentiation was not sharp. The use of 40 and 50 ug/g of b.w. of BUdR produced a similar percentage of differentiated metaphases with a higher mitotic index in animals treated with 40 ug/g of b.w. of BUdR. Mice which received 50 ug/g of b.w. of BUdR also showed a higher number of chromatid aberrations. Based on these results, 40 ug/g of b.w. of BUdR was selected as a standard dose.

D. Effect of Various Concentrations of FUdR on Percentages of Differentiated Metaphases and SCEs

Effects of various doses of FUdR on the mitotic index, number of differentiated metaphases and SCE along with 40 ug/g of b.w. of BUdR and 1 ug/g of b.w. of deoxycytidine given on alternate hours were evaluated using 13-17 hr schedule. The results are presented in Table 4. Without FUdR, the serial injections of BUdR failed to produce well

Table 3: Effects of Various Concentrations of BUdR on Mitotic Index, Percentage of Differentiated Metaphases and the SCEs in C3H Male Mice.

| No. of Animals | Conc. of BUdR (ug/g) | Mitotic Index | Percentage of Differentiated Metaphases | SCE |
|---|---|---|---|---|
| 2 | 30 | 7.20 | 18 | 1.45 |
| 2 | 40 | 6.85 | 23 | 1.92 |
| 2 | 50 | 5.60 | 25 | 2.20 |
| 2 | 60 | 4.20 | 28 | * |

*Cells appeared lightly stained

Table 4: Effects of Various Concentrations of FUdR on Percentage of Differentiated Metaphases and SCEs in Male C3H Mice.

| No. of Animals | Dose of FUdR (ug/g) | Percent of Differentiated Metaphases | SCE |
|---|---|---|---|
| 2 | 0 | * | ** |
| 2 | 2 | 24.67 | 2.02 |
| 2 | 4 | 26.65 | 2.00 |
| 2 | 8 | 28.93 | 3.32 |
| 2 | 10 | 29.24 | 3.44 |
| 2 | 16 | 34.41 | 3.81 |

*Very poor differentation

**SCE could not be counted

differentiated metaphases. This was perhaps the result of the rapid metabolic degradation of BUdR. As the concentration of FUdR increased, percentages of differentiated metaphases as well as the SCE levels were increased. Since FUdR was necessary for the BUdR dependent chromatid differentiation, it was decided to determine a dose level which would maximize the number of differentiated metaphases and minimize background SCE. To compare the effectiveness of various doses, the SCE levels of each dose were divided by the percentages of differentiated metaphases. Based on these values 2 ug/g of FUdR was considered as the effective dose.

Higher doses of FUdR were also tried in order to determine their effects on bone marrow cells. Two C3H male animals were given 40 ug/g of b.w. of BUdR and two animals received 20 ug.g of b.w. of FUdR with 40 ug/g of b.w. of BUdR in 9 hourly injections. Both doses resulted in very few interphase and metaphase cells.

E. Mitotic Indices and Percent of Differentiated Metaphases in Femur and Tibia in C3H and C57BL Males

To compare the suitability of marrow cells from femur and tibia, mitotic indices, percentages of differentiated and SCEs were analyzed using 13-17 hr injection schedule with previously described doses of BUdR, FUdR, deoxycytidine and colchicine. The results are presented in Table 5.

Although no significant differences were found in mitotic indices and percentages of differentiated metaphases

Table 5: Mitotic Indices, Percentages of Differentiated Metaphases and SCEs in Cells Collected from Femur and Tibia of C3H and C57BL Male Mice Using 13-17 hr Injection Schedules and at Room Temperature (22-24°C).

| No. and Strain of Animals | | Mitotic Index ± SEM* | | Percent of Differentiated Metaphases ± SEM | | |
|---|---|---|---|---|---|---|
| | | Femur | Tibia | Femur | Tibia | SCE±SEM |
| 4 | C3H | 8.22±0.97 | 7.57±0.67 | 19.56±1.62 | 21.33±2.36 | 1.95±0.20 |
| 4 | C57BL | 7.31±0.80 | 6.50±0,75 | 26.68±1.30 | 24.00±1.78 | 2.05±0.22 |

*SEM: standard error of mean

in cells from femur and tibia, the preparations from the tibia had consistently less cellular debris and blood contamination.

Most of the time, the temperature of the room where the animals were injected with BUdR and FUdR was between 22-30°C. In one instance involving six C57BL males the room temperature rose to 34°C and remained there until after the mice were sacrificed. Mitotic indices, percent of differentiated metaphases and SCEs were analyzed in marrow cells from the femur and the tibia for two injection schedules: 13-15 hr and 13-17 hr (Table 6). The 13-17 hr injection schedule at high room temperature (34°C) and high humidity (R.H. 70%) produced a significantly ($p < .01$) higher number of differentiated metaphases and SCEs than at normal (24-30°C) room temperature (Table 5). The mitotic index however remained unaffected. Femur and tibia did not show any significant differences in mitotic indices and the percentage of differentiated metaphases. Although some variability between individuals in the number of differentiated metaphases was observed, a specific set of conditions produced uniform SCE values. Kato (1980) has reported that culturing Chinese hamster cells at temperatures above 39°C enhanced SCE formation. Similarly, temperature SCE induction in Chinese hamster V-79 cells has been reported by Speit (1980).

Table 6: Mitotic Indices, Percentage of Differentiated Metaphases and SCEs in Cells Collected from Femur and Tibia of C57BL Male Mice Using 13-15 and 13-17 hr Injection Schedules and at High Room Temperature (34°C) and High Humidity (70%).

| No. and Strain of Animals | Time of Injection Schedule | Mitotic Index ± SEM* | | Percent of Differentiated Metaphases ± SEM | | SCE±SEM |
|---|---|---|---|---|---|---|
| | | Femur | Tibia | Femur | Tibia | |
| 2 C57BL | 13-15 | 5.10±0.10 | 5.55±0.20 | 23.00±0.20 | 18.50±0.50 | 1.90±0.28 |
| 4 C57BL | 13-17 | 5.86±0.74 | 7.07±0.43 | 39.77±3.15 | 41.14±5.92 | 3.18±0.08 |

*SEM: standard error of mean

F. Mitotic Indices, Percentage of Differentiated Metaphases and SCEs in Laboratory Maintained Wild Mice

Four wild mice, maintained in the laboratory over a period of six months were tested for the applicability of the system and also to determine the base level of SCEs in these animals. These mice were subjected to 9 hourly injections of 40 mg/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and 1 ug/g of b.w. of deoxycytidine (on alternate hours) with 5 ug/g of b.w. of colchicine being injected on the 13th hr for 4 hr and were maintained at normal room temperature.

Table 7 shows the mitotic indices, percentages of differentiated metaphases and SCEs in laboratory maintained wild male mice. The average mitotic index and SCE values in these mice were 4.46 and 2.18 respectively, while similar values in the control C57BL were 5.79 and 1.85. The percentage of differentiated metaphases in wild animals was considerably lower than in the controls (14.75 vs 22.00). Because wild mice gave this low percentage of differentiated metaphases, further improvements in the procedure were sought.

G. Effects of Number of Injections

With the dose levels the same, the number of hourly injections was increased to 10 and 11. The effects of 12, 45 min interval injections and 18 half hourly injections were also examined.

No differences were observed between 9 and 10 hourly injections. Other injection schedules produced a higher

Table 7: Mitotic Indices, Percentage of Differentiated Metaphases and SCEs in Inbred and Laboratory Maintained Wild Male Mice Using 13-17 hr Injection Schedule.

| Animals | Mitotic Index | Percentage of Differentiated Metaphases | SCE |
|---|---|---|---|
| C57BL | 5.79 | 22 | 1.85 |
| Dover (wild) | 4.65 | 13 | 1.87 |
| Houle (wild) | 4.36 | 15 | 2.40 |
| Laramie (wild) | 4.40 | 16 | 2.32 |
| Comartin (wild) | 4.41 | 15 | 2.13 |
| Average values for wild mice | 4.45 | 14.75 | 2.18 |

number of SCEs but the mice showed more stress. Moreover, the metaphase chromosomes showed stickiness and poor spread. Therefore, increasing the number of hourly injections or shortening the injection intervals did not improve the results.

H. Effect of Deoxycytidine on the Percentage of Differentiated Metaphases

The next approach was to continue the 9 hourly injection schedule but evaluate the effects of deoxycytidine. Four C3H male mice were given 9 hourly injections of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and bihourly injections of 1 ug/g of b.w. of deoxycytidine with 5 ug/g of b.w. of colchicine on the 13th hr for 4 hr. The other group of four animals were given exactly the same treatment but deoxycytidine was excluded. In animals which received deoxycytidine, the average percentage of differentiation was 20.00, but the corresponding value in mice without the treatment of deoxycytidine was 25.00. The mitotic indices in deoxycytidine treated and untreated animals were 6.50 and 5.95 respectively. The SCE values in mice which received deoxycytidine was 2.04. The conclusion from this experiment was that deoxycytidine might be counteracting the effects of FUdR and hence, reducing the incorporation of BUdR molecules into the newly synthesized DNA strands.

Further tests were done with wild male mice to evaluate the effects of the absence of deoxycytidine on

the number of differentiated metaphases. Four laboratory maintained male wild mice were given 9 hourly injections of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR without any deoxycytidine. The average percentage of differentiated metaphases was 18. Mice with lower body weights ( < 15 g) failed to produce the required number of differentiated metaphases. Since wild mice in general were of lower body weight, the application of the present injection schedule to wild mice was not very suitable.

I. Effect of Different Injection Schedules on Mitotic Indices, Percentage of Differentiated Metaphases and SCEs in Laboratory Maintained Inbred and Wild Mice

The effectiveness of other injection schedules was explored. Table 8 shows the mitotic indices, percentage of differentiated metaphases and SCEs in inbred and laboratory maintained wild mice at different time schedules, with 9 hourly injections of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and 5 ug/g of b.w. of colchicine.

The results show that the percentage of differentiated metaphases and SCEs increased when the animals were sacrificed at later times. The colchicine treatment was reduced from 4 hr to 3 hr after the 21st hr time, on the assumption that the cells had undergone two replication cycles and enough metaphases after the 3 hr of colchicine treatment could be obtained.

The highest mitotic index was obtained when the

Table 8: Mitotic Indices, Percentage of Differentiated Metaphases and SCEs in Inbred and Laboratory Maintained Wild Male Mice Using Various Injection Schedules.

| Group | Injection Schedule | No. and Strain of Animals | Mitotic Index | Percentage of Differentiated Metaphases | SCE |
|---|---|---|---|---|---|
| I | 13-17 hr | 2 C3H | 7.06 | 23.00 | 1.60 |
| | | 2 wild | 6.81 | 20.00 | 1.75 |
| II | 17-21 hr | 2 C57BL | 3.82 | 25.00 | 1.83 |
| | | 2 wild | 4.70 | 28.12 | 2.00 |
| III | 21-24 hr | 2 C57BL | 1.88 | 36.11 | 2.10 |
| | | 2 wild | 2.02 | 30.50 | 2.21 |
| IV | 24-27 hr | 2 C57BL | 1.72 | 45.00 | 2.10 |
| | | 2 wild | 2.31 | 38.76 | 2.27 |
| V | 27-30 hr | 2 C3H | 2.23 | 53.80 | 3.00 |
| | | 2 wild | 3.12 | 42.85 | 2.90 |

animals were sacrificed on the 17th hr following four hr of colchicine treatment. The wild mice showed lower mitotic indices for all injection schedules except for the 13-17th hr schedule. The percentage of differentiated metaphases was slightly higher in inbred strains than wild mice. When animals were sacrificed at later times, there was an increase in the percentage of differentiated metaphases and SCE frequencies. However, animals in Group V produced well differentiated metaphases with no partially or incompletely differentiated metaphases which were encountered in earlier time schedules.

Based on these results, it was concluded that the 27-30 hr schedule was best, because this schedule gave the highest number of differentiated metaphases, required number of mitotic figures and the minimum number of incompletely differentiated metaphases in inbred and wild mice. The revised protocol for freshly caught wild mice, therefore, was to inject 9 hourly doses of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and 5 ug/g of b.w. of colchicine on the 27th hr for 3 hr. The earlier time schedule, although suitable for inbred mice, which in general were heavier, was not suitable for the lighter wild mice. The latter gave better results with the 27-30 hr schedule.

J. Baseline SCEs in Certain Inbred Strains and Laboratory Maintained Wild Male Mice

Using the revised protocols (9 hourly injections of

40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and 5 ug/g of b.w. of colchicine on the 27th hr for 3 hr), SCEs were determined in certain inbred strains and wild mice in order to establish the baseline SCEs (Table 9).

C3H animals gave the lowest baseline SCE value. The DBA and $F_1$ animals showed somewhat higher SCEs over the C3H and C57BL mice. Female mice showed considerably higher SCE values than did the male counterparts. This was observed in all strains of mice that were examined.

The frequency of SCE in laboratory maintained male mice was simialr to that seen in control male inbred for both time schedules (13-17 hr and 27-30 hr) examined. Table 20 (Appendix II) shows the SCE values of individual male mice maintained in the laboratory for 6-9 months.

K. Testing the System with MMC

Mitomycin-C (MMC), a bifunctional alkylating agent which cross links with the complimentary strands of DNA, is a known inducer of SCE (Allen and Latt, 1976; Kram et al., 1979).

Using the present procedure MMC showed a dose dependent incerase in SCEs (Table 10). A statistically significant difference ($p < 0.05$) was observed between the SCE values of the animals not treated with MMC and animals treated with 1 ug/g of b.w. of MMC. These experiments were conducted with 9 hourly injections of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and 5 ug/g of b.w. of colchicine

Table 9: Baseline SCEs in Certain Inbred Strains of Laboratory Maintained (6-9 month) Male Wild Mice.

| Strains | Total No. of Mice Examined | Mean SCE±SEM (per animal) |
|---|---|---|
| C3H males | 19 | 3.42±0.07 |
| C57Bl males | 17 | 3.62±0.08 |
| DBA males | 3 | 3.97±0.13 |
| F1 (C3H male x C57Bl female) males | 4 | 4.13±0.17 |
| C3H females | 4 | 5.09±0.03 |
| C57Bl females | 4 | 5.89±0.15 |
| F1 (C3H males x C57Bl female) females | 2 | 6.16±0.00 |
| Laboratory maintained wild males | 13 | 3.46±0.12 |

Table 10: Effects of MMC on Chromosome Morphology and SCEs in C3H and C57BL Male Mice With 13-17 and 27-30 hr Injection Schedules.

| No. & Strain of Animals | Injection Schedule | Dose of MMC (ug/g) | Percentage of Fragmented Metaphases | SCE |
|---|---|---|---|---|
| 2 C3H | 13-17 | 0 | 0 | 1.60 |
| 2 C3H | 13-17 | 1 | 2 | 2.50 |
| 2 C3H | 13-17 | 2 | 4 | 3.10 |
| 2 C3H | 13-17 | 3 | 10 | 4.76 |
| 2 C3H | 13-17 | 4 | 25 | no SCE was counted |
| 2 C3H | 13-17 | 8 | Complete suppression of mitotic index | |
| 2 C3H | 27-30 | 0 | 0 | 3.00 |
| 2 C57BL | 27-30 | 0 | 0 | 3.20 |
| 2 C3H | 27-30 | 2 | 0.5 | 5.45 |
| 2 C57BL | 27-30 | 2 | 1.0 | 5.60 |

at the 13th hr for four hr. The animals were sacrificed on the 17th hr.

The effects of MMC were also evaluated for the 27-30 hr time schedule. When 2 ug/g of b.w. of MMC was injected 24 hr prior to the first injections of BUdR/FUdR, the SCE values in C57BL and C3H male mice were 5.60 and 5.45 respectively. In the control C57BL and C3H mice not treated with MMC, the SCE values were 3.20 and 3.0 respectively (Table 10). A higher number of fragmented metaphases was observec in mice treated with MMC and sacrificed at the 17th hr as compared to the 27th hr. MMC higher than 4 ug/g of b.w. was found extremely damaging to murine metaphase chromosomes.

## 4. CONCLUSIONS

Murine bone marrow cells provide a good test tissue for SCE analysis because of their high mitotic index. From the sister chromatid exchange data, it appears, that the in vivo bone marrow cell cycle in mice under the existing conditions (i.e. when treated with BUdR, FUdR) may be similar to that observed in Chinese hamster cells by Fremuth et al., (1976).

A procedure involving 9 hourly injections of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and 5 ug/g of b.w. of colchicine is sufficient to give an adequate number of

differentiated metaphases for SCE analysis. The 13-17 hr time schedule was found suitable for heavier inbred mice (mice weighing over 25 g), but not for wild mice or mice weighing less than 20 g. The 27-30 hr time schedule gave the best results here and acceptable results for the heavier inbred mice. Deoxycytidine was found unnecessary whereas FUdR was necessary for differential staining. Hanks balanced salt solution at pH 7.0 was a suitable solvent for BUdR, FUdR and deoxycytidine. The neutrality of the pH however appeared extremely important for a high incorporation of BUdR. All solutions, particularly BUdR and FUdR solutions required protection from light in order to prevent photolysis. Potassium chloride (0.075M KCl) and methanol/glacial acetic acid fixative were found suitable for bone marrow cells. Cells collected from femurs and tibia gave similar results, however, the preparations from the tibia were much clearer and did not contain any cellular debris. Tibia was the bone of choice even though the amount of cells collected from this bone was small.

C3H mice gave the lowest baseline SCEs, while C57BL produced a higher number of differentiated metaphases. The mitotic index appeared somewhat higher in C3H animals. DBA and $F_1$ mice gave higher SCE values than the other two inbred strains.

Female mice showed a higher number of differentiated metaphases and a higher frequency of SCEs compared to

that of male mice. A sharp chromatid differentiation was observed in cells from female mice. A greater variability in the SCE values of female inbred mice was also observed compared to the male inbreds.

Wild mice maintained in the laboratory over a period of six months, gave SCE values similar to those of control inbred strains.

High temperature (34°C) and high humidity (70%) during the injection period seemed to have a strong effect on the percentage of differentiation and SCEs without any appreciable effect on mitotic index and chromosomal aberrations.

# CHAPTER III

# CHROMOSOME AND CHROMATIN ABERRATIONS ASSOCIATED WITH THE TEST SYSTEM

## 1. INTRODUCTION

The types of chromosome damage which can be cytologically distinguished at metaphase are divided into two main groups: 1) chromosome type- when the two chromatids are affected at the same locus. 2) chromatid type- when only one chromatid is affected at a given locus (Savage, 1976).

The types of aberrations that can be observed include gaps, breaks, deletions, fragments, radial exchange figures, pulverized metaphases, complex structural rearrangements, dicentrics and rings (Bostock and Sumner, 1978). Gaps or achromatic regions are unstained areas of the chromosomes without any visible chromatin material. They appear as wide as a chromatid. Breaks on the other hand, are chromatic and are not aligned with the chromatid (Bostock and Sumner, 1978).

Chromatid aberrations can be induced by physical and chemical mutagnes (Perry and Evans, 1975; Russell, 1979; Renner, 1979; Douglas et al., 1980). The type of aberration depends on the stage of the cell cycle and the nature of the agents (Evans, 1962; Evans and Scott, 1969; Bender et al., 1974; Wolff, 1978; Wolff, 1981a). The stages susceptible to chromosomal aberrations include, metaphase, anaphase, telophase, (mitosis), early pre-DNA synthetic stage ($G_1$), and

the beginning of DNA synthesis (S). However, exposure of cells to these agents in the later stages of $G_1$, S and the post-DNA synthetic period ($G_2$) result in chromatid aberrations (Bender et al., 1974). Exposure during the prophase stage may result in a sub-chromatid type of aberration (Brinkley and Humprey, 1969, Evans and O'Riordan 1975; Bender et al., 1974).

Korte (1980) has shown in Chinese hamsters, that cyclophosphamide produces chromatid breaks, isochromatid breaks and chromatid translocation whereas aflatoxin $B_1$ produces only chromatid breaks, isochromatid breaks but no chromatid translocation; aflatoxin $G_1$, on the other hand, produces chromatid breaks.

The induction of sister chromatid exchange differentiation requires a series of intraperitoneal injections of BUdR, FUdR, and in some cases, deoxycytidine (Bauknecht et al., 1977; Roszinsky-Kocher et al., 1979; Allen and Latt, 1976a). BUdR and FUdR have been reported as cell cycle specific mutagens (Aebersold, 1976, 1979). BUdR has been known to cause chromosomal aberrations in cultured mammalian cells (Hsu and Somers, 1961; Dewey and Humphrey, 1965; Huang, 1967). FUdR has been recognized as being specific in the cause of aberrations such as achromatic gaps (Bender et al., 1974).

Colchicine, which is used to arrest the cells at the metaphase stage, does not appear to cause any chromosomal

aberrations (Hughes, 1952; Eigsti and Dustin, 1955; Tschimoto and Matter, 1979).

Mitomycin-C (MMC) is a mutagenic (Szybalski, 1958; Iijima and Hagiwara, 1960; Carrano et al., 1978). It is commonly used as a test compound in evaluating the sensitivity of in vitro and in vivo SCE inducing systems (Latt 1974a; Latt et al., 1975; Perry and Evans, 1975; Allen and Latt 1976a,b; Galloway, 1977; Carrano et al., 1978; Kram and Schneider, 1978; Ishii, 1981). MMC has also been known as a clastogenic agent (Merz, 1961; Cohen and Shaw, 1964; Nowell, 1964).

There are some studies which have evaluated the frequency of chromosomal aberrations induced by the joint treatment of BUdR, FUdR, colchicine and MMC under conditions that differed from those used in this study.

Roszinsky-Köcher and Röhrborn (1979), using serial injections of BUdR and FUdR in Chinese hamster cells and colchicine to arrest the metaphases, have shown that control animals show 3% of the metaphases with structural aberrations such as gaps, breaks and fragments. They have also further demonstrated that cyclophosphamide, an indirect mutagen, produces exchanges, deletions and multiple aberrations at a higher dose. Kram and Schneider (1978) used the intravenous infusion method of BUdR substitution in different strains of mice and have shown that the frequency of chromosomal aberrations in untreated (no BUdR, no MMC) C57BL/6J

and AKR/J was 0.105 and 0.12 per cell, respectively. Their results indicate that MMC produced 0.39 chromosomal aberrations per metaphase cell in C57BL/6J and 0.55 per metaphase cell in AKR strains. The type of chromosomal aberrations induced by MMC included: breaks, gaps, fragments, dicentrics, tri-radials and quadri-radials. The tri- and quadri-radial figures appeared as three or four armed chromosomes respectively. The predominant aberrations were gaps and breaks. In an in vitro study with CHO cells (Perry and Evans, 1975) it was shown that MMC induced chromosomal aberrations in 0.18% of the chromosomes and chromatid aberrations in 0.90%. MMC and BUdR together in metaphase-1 (M-1) cells caused 0,54% of chromosomal aberrations and 1.73% of the chromatid aberrations. In M-2 cells the two chemicals induced chromosomal aberrations in 0.54% of chromosomes and chromatid aberrations also in 0.54% of the chromosomes examined. The chromosomal aberrations included: dicentrics, rings, deletions, gaps, and breaks. The chromatid aberrations included: rings, isochromatids and interchanges. Brøgger (1979) has indicated that the unstable aberrations like breaks, fragments, rings, and dicentric chromosomes are mainly seen in first mitosis (M-1). The same author has also suggested that chromatid gaps and attenuations caused by alkylating agents are due to folding defects of chromosomal fibres. This in fact may mean that a target(s) other than DNA is involved in some of the above

types of aberrations.

Tsuchimoto and Matter (1979) have studied the cytogenetic effects of FUdR, and BUdR in Chinese hamster bone marrow cells following intraperitoneal injections of BUdR and FUdR. Their study shows that FUdR with or without BUdR induces micronuclei and chromosome damage, whereas BUdR alone does not.

This segment of the study was concerned with the types and frequencies of chromosomal aberrations that were observed in inbred and wild mice due to BUdR, FUdR, deoxycytidine, colchicine and MMC.

## 2. MATERIALS AND METHODS

The general proceudre for collecting and processing of the bone marrow cells was described in Chapter II.

In most cases, the aberrations were scored on slides stained for SCE analysis using 5% Fisher's or Merck's Giemsa in Gurr's buffer (pH 6.8). In some instances, slides were stained with 4% Gurr's R-66 Giemsa stain in Sörenson buffer for 8 min and also with 2% Aceto-orcein and with Feulgen stain. Formulae are given in Appendix 1.

About 1000 cells were examined for chromatin aberrations, One hundred metaphases per animal were analyzed for estimating the frequency of chromosomal/chromatid aberrations. All aberrations were scored by light microscopy using the

100 x oil immersion objective. All chromosomal/chromatid aberrations were expressed in terms of the number of differentiated metaphases counted.

## 3. RESULTS AND DISCUSSION

### A. The Effect of Colchicine on Chromosomal Anomalies in C3H and Wild Male Mice

The effects of colchicine on bone marrow metaphases of C3H and wild male mice are given in Table 11. The types of anomalies observed were Y chromatid separations, achromatic gaps, chromatid exchanges, twisted chromatids, condensed (heteropyknotic) chromosomes and chromosomes showing an unequal chromatid length. The Y chromatid separations were the predominant type of aberrations. These were commonly seen in the early pro-metaphases. An average of 0.13% of the metaphases showed chromosomes with unstained (achromatic) lesions or gaps. Brøgger (1979) has suggested that achromatic gaps may result from a type of incomplete folding of the chromosome fibres. Fig. 5 shows a metaphase spread with acentric fragments. It is difficult to explain the reasons for the Y chromatid breakage. Chemicals capable of affecting the function of the centromeric heterochromatin have been reported as causing nondisjunction (Vig, 1977).

### B. Chromosonal Aberrations in C3H and C57BL and Wild Male Mice

Table 12 shows the type and frequencies of chromosomal

Table 11: Effects of Colchicine (5 ug/g of b.w.) for 4 hr on the Percentage of Chromosomal Anomalies in Male Inbred and Wild Mice.

| Strains and No. of Mice | C3H (4) | Wild (4) |
|---|---|---|
| Total metaphase counted | 320 | 360 |
| Y chromatid breaks or acentric fragments | 4.0 | 6.4 |
| Unequal chromatid ratios | 2.8 | 2.5 |
| Acrocentric associations | 0.10 | 0.30 |
| Twisted chromatid of one chromosome making a loop form shape | 2.80 | 3.74 |
| Achromatic lesions | 0.30 | 0.32 |
| Chromatid exchanges | 0.00 | 0.27 |
| Condensed medium sized chromosome | 0.30 | 0.57 |
| Condensed small sized (Y) chromosome | 0.27 | 0.30 |
| Small mininuclei | 0.00 | 0.63 |

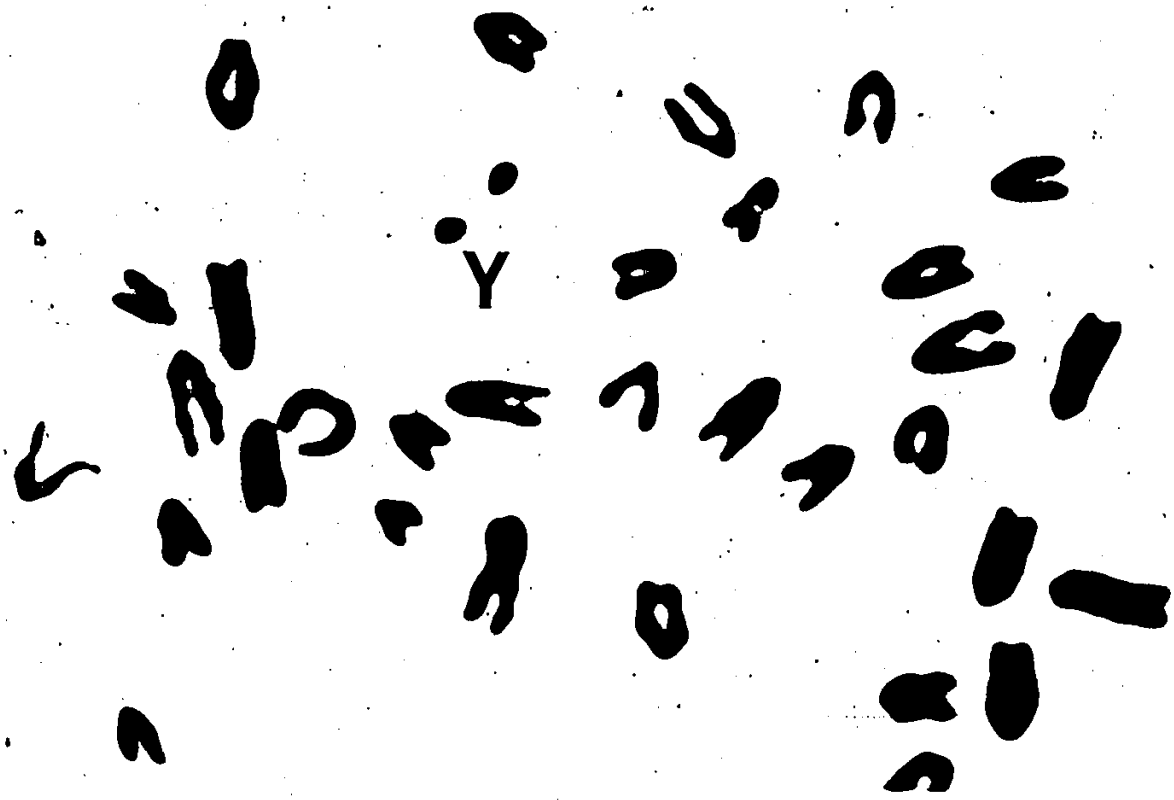


Fig. 5. Acentric fragments of Y chromosome in a spread (x5,700; Giemsa stain).

Table 12: Percentage of Chromosomal Aberrations Observed in C3H, C57Bl and Wild Male Mice Using 13-17 hr Injection Schedule.

| Animals | 2 C3H | 2 C57Bl | 3 Wild |
|---|---|---|---|
| Total metaphases counted | 200 | 200 | 300 |
| Y chromatid separation | 2 | 2 | 4 |
| Fragmented metaphase chromosomes | 0 | 0 | 1 |
| Deletions | 1 | 1 | 2 |
| Acrocentric association | 1 | 1 | 1 |
| Mininuclei | 0 | 0 | 0.5 |
| Achromatic lesions | 0.5 | 1 | 2 |
| Metacentric-like differentiated chromosomes | 0.5 | 1 | 1 |
| A small chromosome (Y) deeply stained in a nondifferentiated metaphase | 2 | 1 | 2 |
| A small chromosome (Y) with both chromatids stained in a differentiated metaphase | 1 | 0 | 1 |

aberrations in C3H and C57BL and wild male mice following 9 hourly injections of 40 ug/g of b.w. BUdR, 2 ug/g of b.w. FUdR and 1 ug/g of b.w. deoxycytidine (on alternate hours) with 5 ug/g of b.w. colchicine given on the 13th hr following the first injections of BUdR, FUdR and deoxycytidine for four hr. No common anomalies were observed. Rare anomalies include: metacentric differentiated (Fig. 6) and non-differentiated chromosomes (Fig. 7), a small chromosome differentially stained in a metaphase (Fig. 8), a small chromosome with both chromatids deeply stained in a differentiated metaphase (Fig. 9). In a metaphase plate where the metacentric-like chromosome was found, there were only 39 acrocentric chromosomes observed. Therefore, the metacentric chromosomes probably arose from the fusion of two acrocentric chromosomes at the centromere. Similar observations have been reported in male DDY mice fed on a diet containing 500 ppm monomeric acrylamide for 3 weeks. The spermatogonial cells in the treated mice showed chromatid exchanges and metacentric chromosomes. The frequencies of these aberrations were low. It has been suggested by the author that the metacentric chromosomes occurred due to Robertsonian translocation.

The small chromosome which was found in rate cases, out of synchrony in its differential staining pattern from the rest of the chromosomes, was identified as the Y chromosomes by the absence of C-banding since the murine Y chromosome

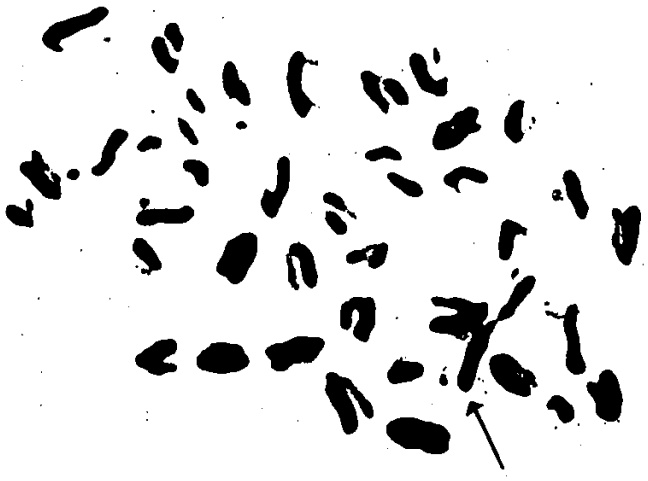

Fig. 6. Differentiated metacentric chromosome in a differentiated metaphase plate (x4,000; Giemsa stain).

Fig. 7. A non-differentiated metacentric chromosome in a non-differentiated metaphase plate (x4,000; Giemsa stain).

Fig. 8. Metaphase showing a chromosome with differentially stained chromatids (probably Y chromosome) (x5,000; Giemsa stain).

Fig. 9. A differentially stained metaphase showing a chromosome (probably Y) with two chromatids equally stained (x4,000; Giemsa stain).

lacks C-banding chromatin. In another metaphase this chromosome appeared to show centromeric disintergration. A Y chromosomal aberration has been reported in a worker exposed to dibromochloropropane (Kapp et. al., 1979).

C. The Effect of BUdR and FUdR on Chromosomal Aberrations

Animals received 9 hourly injections of BUdR/FUdR with 5 ug/f of b.w. of colchicine on the 13th hr and were sacrificed 4 hr later. BUdR did not appear to cause any substantial chromosomal aberrations. The chromosomal aberrations observed with various doses of FUdR are presented in Table 13. In some metaphases, a chromosome (Y) appeared as two fragments, possibly due to the loss of their centromeric portion of the chromatin. It should be pointed out that Y chromosome aberrations have also been observed in cells treated with colchicine alone. The frequency of these particular aberrations appeared to increase as the doses of FUdR increased. Other aberrations which showed an increase with the addition of more FUdR at each dose were tri- and quadri-radials. These figures appeared as three or four armed chromosomes.

Other chromatid aberrations such as achromatic lesions, chromatid breaks, deletions, translocations and dicentrics appeared between 1-2% in frequency. In a few of the metaphases (about 0.5-1%) extended chromatin filament from the centromere was observed. Schreck et. al., (1979) have reported biarmed chromosomes with extended centromeric

Table 13: Percentage of Chromosomal Aberrations Observed in C3H Male Mice Due to Different Doses of FUdR Using a 13-17 hr Injection Schedule.

| Dose of FUdR (μ/g) | 2 | 4 | 8 | 10 | 16 |
|---|---|---|---|---|---|
| No. of Animals | 2 | 2 | 2 | 2 | 2 |
| Total No. of differentiated metaphases counted | 200 | 200 | 200 | 200 | 200 |
| Y chromatid separation | 4.5 | 5 | 9 | 9.50 | 11.5 |
| Chromatid breaks | 0.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Achromatic lesions | 0.0 | 0.0 | 2.0 | 1.5 | 2.0 |
| Deletions | 0.0 | 0.0 | 0.0 | .30 | 0.5 |
| Tri-and quadriradials | 0.0 | 0.0 | 3.0 | 3.0 | 4.0 |
| Acrocentric association between chromosomes | 2.0 | 3.0 | 2.0 | 2.0 | 3.0 |
| Dicentrics | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Translocations | 0.0 | 0.0 | 1.0 | 1.0 | 1.0 |
| Extended chromatin filament from the centromere | 0.5 | 0.0 | 0.0 | 1.0 | 1.0 |

heterochromatin in bone marrow cells of CBA male mice treated with high doses (225 ug/g and 400 ug/g) of acetylamine fluorene (AAF) following BUdR treatment. These workers have also shown multiple centromeric association due to AAF.

Other types of anomalies found were: metacentric-like chromosomes (0.2%), small chromosomes differentially stained in a non-differentiated metaphase (1-3%) and a small chromosome with both arms stained in a non-differentiated metaphase (0.1%). Two female C3H mice were also tested for the effect of high doses of FUdR (10 ug/g) using the 13-17th hr injection schedule. The types of aberrations found were minichromosomes, chromatid separations (probably X chromosomes), extended centromeric chromatin, achromatic gaps, deletions and dicentrics. Female mice showed 3 times the number of structural chromatid aberrations seen in males treated with similar doses of FUdR.

D. Effect of Temperature and Humidity on Chromosomal Aberrations

Animals which received 9 hourly injections of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and 1 ug/g of b.w. of deoxycytidine (on alternate hours) at normal room temperature (22-23°C) and at high room temperature (34°C) and humidity (70% R.H.) were analyzed for chromosomal aberrations.

There was no significant difference in frequencies of aberrations in the animals subjected to high (34°C) and normal (22-24°C) room temperatures (Table 14).

Table 14: Percentage of Chromosomal Aberrations Observed in C57Bl and Wild Mice at Normal Room Temperature (22-24°C) and C57Bl Male Mice at High Room Temperature (34°C) and High Humidity (70°).

| No. and Strain of Animals | Normal Room Temp. (22-24°C) 4 C57Bl | 4 Wild | High Room Temp. (34°C) 4 C57Bl |
|---|---|---|---|
| Total no. of metaphases counted | 400 | 400 | 400 |
| Metacentric-like differentiated chromosomes | 0.5 | 0.5 | 0.5 |
| A small chromosome (Y) with borh chromatids deeply stained in a non-differentiated metaphase | 0.75 | 0.5 | 0.0 |
| A small chromosome (Y) with both chromatids stained in a differentiated metaphase | 0.5 | 0.25 | 0.25 |
| Acrocentric association | .25 | 1.0 | 0.25 |
| Achromatic lesions | 1.0 | 1.5 | 1.0 |
| Y chromatid separation | 1.0 | 2.0 | 1.0 |
| Chromosomal fragmentation | 0.0 | 0.5 | 0.0 |
| Mininuclei | 0.0 | 0.5 | 0.0 |
| Deletions | 1.0 | 0.5 | 0.0 |

E. Effect of MMC on Chromosomal Aberrations

Table 15 shows the effects of MMC on murine bone marrow chromosomes.

When 2 ug/g of b.w. of MMC were injected using two doses, one at 24 hr prior to and the other at 8 hr prior to the first injections of BUdR/FUdR in C3H males, 4% of the metaphases showed a small chromosomes (probably Y) as being highly pyknotic. Four and 6% of the metaphases showed chromosomes with terminal deletions and whorl arrangements, respectively. Table 15 shows the type and frequencies of aberrations in MMC treated C3H male animals. Chromatid aberrations were the predomonant type. The clustering of chromosomes in the metaphase plates, deletions, breaks and gaps were the other types of aberrations observed. When MMC was injected 24 hr prior to the first injections of BUdR/FUdR, there did not appear to be any differences between the frequency of aberrations in MMC treated and control animals.

When 2 ug/g of b.w. of MMC were injected 24 hr, 13 hr and 6.5 hr before, and 6.5 hr, 13 hr, and 24 hr after the first injections of BUdR/FUdR, similar types of aberrations were observed. However, complex aberrations (Fig. 10) occurred only in mice treated with MMC on the 13th hr following the first injections of BUdR/FUdR and being sacrificed on the 30th hr following 3 hr of colchicine treatment. In general, MMC caused several structural

Table 15: Percentage of Chromosomal Aberrations Observed in C3H Males Treated With MMC.

| | No MMC | MMC (2 ug/g) 24 hr prior to | MMC (2 ug/g) 8 hr prior to | MMC (3 ug/ 8 hr prior to |
|---|---|---|---|---|
| No. of animals | 2 | 2 | 2 | 2 |
| No. of metaphases counted | 200 | 200 | 200 | 200 |
| Centromeric association and clustering of chromosomes | 4 | 6 | 11 | 34 |
| Small chromosome deeply stiained in metaphases | 9 | 5 | 2.3 | 0 |
| An extended chromosome in metaphases | 2 | 0 | 0 | 0 |
| Metacentric-like chromosome | 2 | 2 | 0 | 0 |
| Enlarged centromere | 2 | 1 | 1 | 1 |
| Chromatid bridge | 2 | 0 | 0 | 0 |
| Fragmented metaphases | 0 | 1 | 2.3 | 3.0 |
| Terminal deletions | 0 | 1 | 0 | 2.27 |
| Y chromatid separation | 2 | 2 | 2.3 | 0 |
| Chromatid breaks | 2 | 1 | 2.3 | 3.0 |
| Achromatic gaps | 2 | 2 | 2.3 | 2.27 |

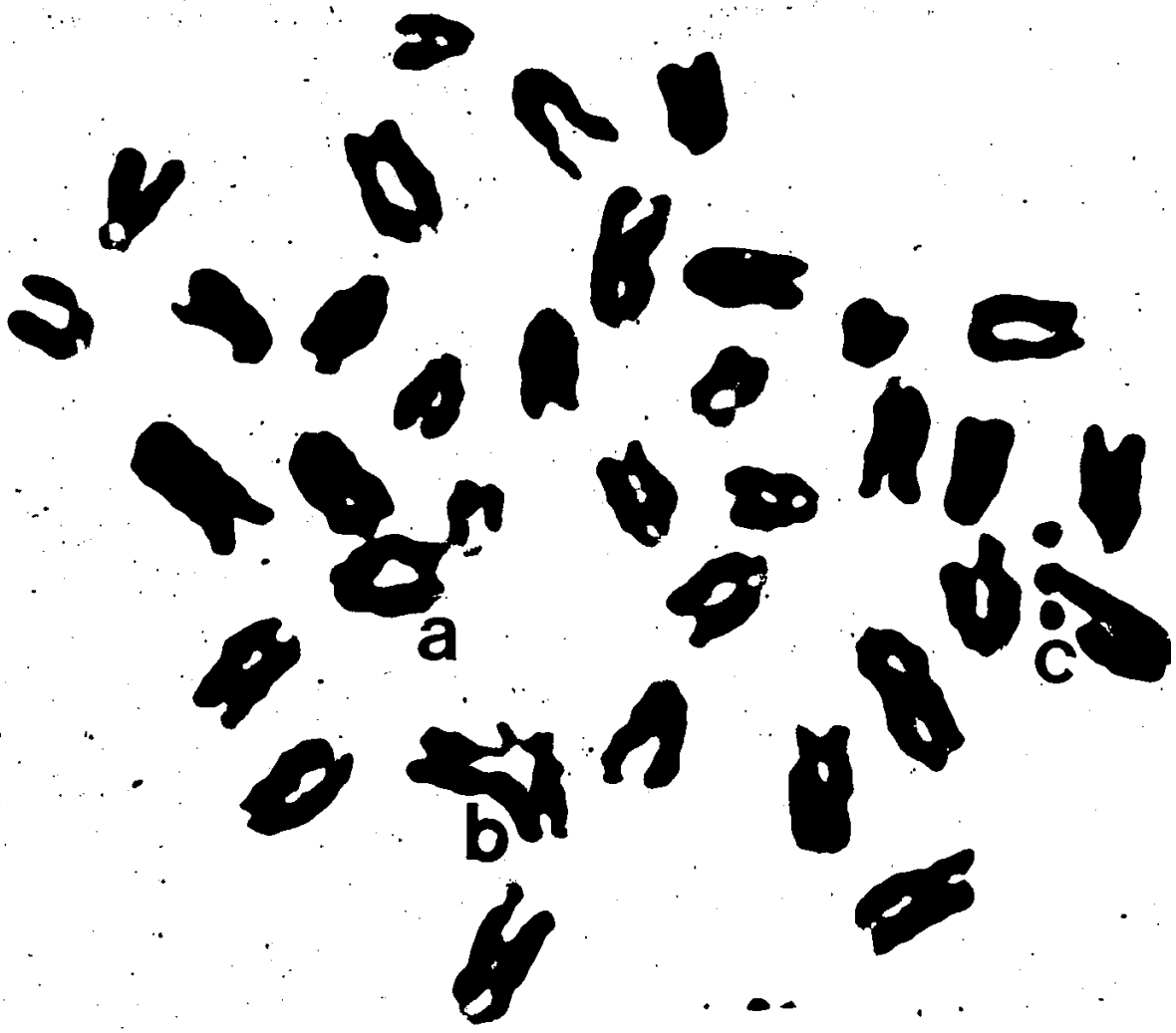


Fig. 10. Metaphase showing multiple aberrations: a) stickiness, b) radial figure and c) fragments (x6,000; Giemsa stain).

aberrations when injected a few hours prior to and a few hours after the BUdR/FUdR treatments.

F. Chromosomal Anomalies in Mice Sacrificed at the 30th Hr

No major aberrations were observed when animals were sacrificed on the 30th hr with 3 hr colchicine treatment on the 27th hr following 40 ug/g of b.w. of BUdR and 2 ug/g of b.w. of FUdR. Occasionally differentiated metacentric metaphases, chromosomes with both chromatids deeply stained and chromosomes with twisted chromatids were observed.

G. Percentage of Micronuclei and Chromatin Bridges in Bone Marrow Cells of C3H and C57BL Males Treated with MMC

When MMC (2.0 ug/g of b.w.) was injected at 24 hr, 13 hr, 6.5 hr before and 6.5 hr, 13 hr and 24 hr after the first injections of BUdR/FUdR in C3H animals with nine hourly injections of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and 5 ug/g of b.w. of colchicine on the 27th hr following the first injections of BUdR/FUdR for 3 hr, a number of chromatin bridges (Fig. 11) were observed. This experiment was extended to C57BL males. These mice showed a greater number of micronuclei (Fig. 12) and no chromatin bridges. Results of these experiments are given in Table 16. These results suggest a strain specific sensitivity to MMC. Similar observations have been reported by Maretoja and Vainio (1979). They analysed the lymphocytes from individuals exposed to styrene and found a considerably higher percentage

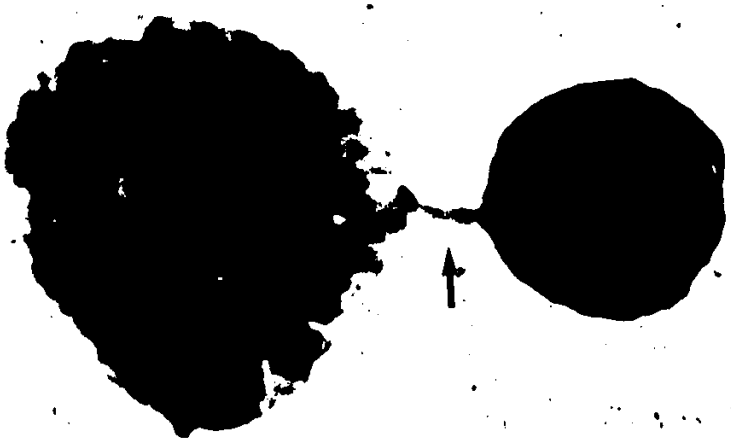

Fig. 11. Chromatin bridge in C3H male animals treated with MMC (x4,700; Giemsa stain).

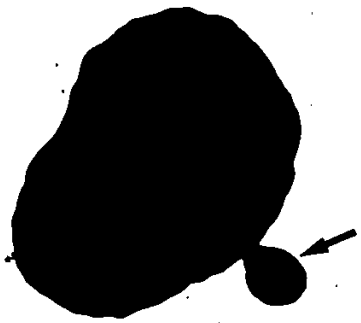

Fig. 12. Micronucleus in MMC treated animals (x4,100; Giemsa stain).

Table 16: Mitotic Indices, Percentage of Micronuclei and Chromatin Bridges in Bone Marrow Cells of C3H and C57BL Males Treated With MMC.

| | | C3H | | | C57BL | | |
|---|---|---|---|---|---|---|---|
| No. of Animals | Time of MMC Injections | Mitotic Index | Micro-nuclei | Chromatin Bridges | Mitotic Index | Micro-nuclei | Chromatid Bridges |
| 2 | No MMC | 3.60 | 0 | 0.53 | 3.80 | 1.04 | 0 |
| 2 | -24 h | 3.30 | 0 | 0.80 | 3.94 | 0.93 | 0 |
| 2 | -13 h | 2.44 | 0.09 | 1.03 | 3.35 | 1.07 | 0 |
| 2 | -6.5 h | 2.90 | 0.00 | 0.34 | 2.36 | 0.98 | 0 |
| 2 | +6.5 h | 2.78 | 0.00 | 0.72 | 1.39 | 1.21 | 0 |
| 2 | +13 h | 3.87 | 0.22 | 0.00 | 2.84 | 1.77 | 0 |
| 2 | +24 h | 4.00 | 0.00 | 0.00 | 2.38 | 0.98 | 0 |

of micronuclei and nuclear bridges compared to controls. Further studies are needed to establish the merits of these cytological parameters in mutagenicity testing programs.

## 4. CONCLUSION

Most of the aberrations observed in metaphase chromosomes are of a chromatid type. Aberrations on treatment with colchicine, BUdR, FUdR are: achromatic gaps, breaks, exchanges and Y chromosome breakage and metacentric chromosomes. In all cases with 13-17 hr time schedule, the aberrations are seen at an extremely low frequency.

FUdR and MMC cause several chromatid aberrations as well as giving rise to the various forms of characteristic exchange (radial) figures. The frequency of aberrations due to MMC depend on the time of exposure. Most of these aberrations are observed in earlier time schedules such as the 13-17 hr one. The 27-30 hr time schedule produced practically no aberrations.

In light of the above results, two major conclusions are reached:

i) During SCE analysis, the chromosomal structural variability, micronuclei, and chromatin bridges can not be used as an alternative testing procedure, because of their low frequencies and because the

wild and inbred mice show no appreciable differences.

ii) The 27-30 hr time schedule is preferred for SCE analysis because it uncovered practically no chromosomal aberrations.

# CHAPTER IV

# APPLICATION OF SCE ANALYSIS TO MICE FOR MONITORING OF ENVIRONMENT

## 1. INTRODUCTION

Environmental pollutants pose a serious problem to man's genome. These agents have been and are continuing to increase steadily. Therefore, there is considerable need for a first line monitoring system for genotoxic agents. The feasibility of using in vivo SCE analysis for such surveillance has been explored. SCEs which involve a reciprocal interchange between DNA molecules of homologous (sister) chromatids in a replicating chromosome, have been described as a sensitive measure of DNA damage resulting from such agents as ultraviolet light (Kato 1973; Wolff et al., 1974), alkylating chemicals (Allen and Latt, 1976a,b; Bauknecht et al., 1977; Latt, 1974; Perry and Evans, 1975; Popescu et al., 1977; Schneider and Gilman, 1979; Stetka and Wolff, 1976; Stetka et al., 1977; Takehisa and Wolff, 1977); industrial chemicals (Maretoja and Vainio, 1979; Roszinsky-Kocher, et al., 1979; Rüdiger et al., 1976; Takehisa and Wolff, 1977; Tsuda et al., 1981); pesticides (Crossen et al., 1978; DeCassia et al., 1981), radioactive isotopes (Gibson and Prescott, 1972) and transforming viruses (Nichols et al., 1978). Carrano et al. (1978) have established a relationship between SCE induction and mutagenesis.

The objectives of this section included the application of SCE technique to wild mice from different geographic locations, and to inbred mice exposed to various conditions including outdoor enclosures. The results were analysed for geographic patterns and the effects of different environmental conditions.

## 2. MATERIALS AND METHODS

### A. Materials

The approach involves assaying SCEs in hemopoietic tissues of femurs obtained from house mouse, Mus musculus domesticus. The mice were either representatives of inbred strains C3H/HeJ, C57BL/6J and DBA/2J or members of distinct natural populations. The inbred mice which were originally obtained from the Jackson Laboratory, Bar Harbor, Maine have been maintained at the University of Windsor for at least 10 generations. The wild mice came from corn cribs in southwestern Ontario. A description of these cribs and the collecting procedure has been given elsewhere (Petras and Topping, 1981).

### B. Procedures

Each mouse received nine hourly intraperitoneal injections of each 40 ug/g body weight of BUdR and 2 ug/g body weight of FUdR. The last injections of FUdR was given at twice this dose. BUdR and FUdR were dissolved in sterile

Hanks balanced solution (pH 7.0). These solutions were then sterilized using a 0.22 um Millipore filter. Colchicine dissolved in sterile 0.95% saline and filter sterilized was injected intraperitoneally at a dose of 5 ug/g of b.w., 27 hr after the first BUdR/FUdR injections. The animals were sacrificed three hr later by cervical dislocation. The collections, preparation and staining of bone marrow cells were the same as described in Chapter II, except that the cells were collected from femur.

All comparisons were analysed statistically using the student's t test.

## 3. RESULTS AND DISCUSSION

The SCE values of the various groups of mice examined are presented in Table 17. Six groups of mice were studied: a) Mice of three inbred strains maintained under typical laboratory conditions, (22°C, 14 hr light; Purina Laboratory Chow and water ad libitum); b) C3H mice maintained under typical laboratory conditions except that corn replaced the laboratory chow; c) Inbred animals housed in outdoor enclosures (100 litre drums covered with hardware cloth) filled with corn; d) Wild mice collected from corn cribs and analyzed for SCEs within 24 hr of capture; e) Wild animals collected from corn cribs 3 to 10 weeks before being examined for SCEs and, f) Wild mice housed in the

Table 17: Mean SCE Values in Inbred and Wild Mice Maintained Under Various Conditions

| Mice | Number | Conditions | Mean SCE/Cell ± SEM (per animal) |
|---|---|---|---|
| Males: | | | |
| C3H/J | 19 | Laboratory - Purina Chow | 3.42±0.07 |
| C57BL/6J | 17 | Laboratory - Purina Chow | 3.62±0.08 |
| DBA | 3 | Laboratory - Purina Chow | 3.97±0.13 |
| C3H/J | 4 | Laboratory - Corn | 3.81±0.20 |
| Wild caught mice | 13 | Laboratory - 9 months | 3.46±0.12 |
| Wild caught mice | 7 | Laboratory - 2 to 10 weeks | 6.09±0.13 |
| Wild caught mice | 49 | Corn cribs in southwestern Ontario | 6.02±0.16 |
| C3H/J | 4 | Enclosures near chemical plant (Windsor) | 5.42±0.17 |
| C3H/J | 18 | Enclosures near petrochemical industry (Sarnia) | 4.83±0.16 |
| Females: | | | |
| C3H/J | 4 | Laboratory | 5.09±0.03 |
| C57BL/6J | 4 | Laboratory | 5.71±0.08 |
| $F_1$(C3H♂ x C57BL♀) | 2 | Laboratory | 6.16±0.01 |
| C3H/J | 5 | Outdoor enclosures (Sarnia) | 5.33±0.37 |
| C57BL/6J | 3 | Outdoor enclosures (Windsor) | 6.11±0.14 |

laboratory for at least nine months before SCE analysis. Mice of the inbred strains C3H and/or C57BL maintained under laboratory conditions were used as a standard or "control" in every experiment and provided a measure of the variability.

Males and females were considered separately because the two sexes in both C3H and C57BL mice gave statistically different ($p < .05$) SCE values. The male SCE values are consistent with those reported by Vogel and Bauknecht (1976) (4 SCE/metaphase for C3H), Dragani et al. (1981) (3.1 SCE/metaphase for C3Hf and C57BL/6J and 3.9 for DBA) and others.

Comparisons of the C3H males with the rest of the males showed significant differences with all groups except the C3H males maintained on corn in the laboratory ($p < .10$) and the wild mice maintained in the laboratory for at least nine months ($p < .20$). The t test results are shown in Table 24 (Appendix II).

These results indicate that diet does not play a major role in the SCE differences observed between the various groups. For instance, inbred mice housed in the laboratory on either corn or Purine Chow had similar SCE values. Also, the genotype of the animals does not have an overwhelming effect on SCEs since wild mice maintained in the laboratory over an extended period have SCE levels very similar to those of C3H mice. This is further supported by the SCE

values of inbred mice housed in outdoor enclosures. These values approach those of wild mice. The differences in the last two groups and also the slight but significant differences between C3H and C57BL males, nevertheless suggest some genetic differences in SCE inducibility.

Short term (less than 10 weeks) maintenance of wild mice in the laboratory had very little effect on the SCE values. A longer period (6 months or more) resulted in a decrease in SCEs. This suggests that the SCE values in newly caught mice are higher because of substances which are absorbed and sequestered by the body for a period of time. The eventual decrease in SCEs could be due to slow degradation or excretion of the chemicals responsible for the higher values. Stetka and Wolff reported similar findings; the incidence of SCEs rose following exposure to alkylating agents like ethyl methanesulfonate, methyl methanesulfonate and cyclophosmamide and then decreased to control levels (Stetka and Wolff 1976).

The SCE values in females were significantly higher than those of males of the same inbred strain under all conditions examined (Table 17). However, inbred females when maintained in outdoor enclosures showed smaller increases in SCEs than their male counterparts. This is not unexpected, since in studies of detoxification by the liver different levels of cytochrome P-450, NADPH cytochrome P-450 reductase and aryl hydroxylases have been reported for the

two sexes. Such differences have been attributed to sex hormones (Watanabe et. al., 1980). As a result of this and to avoid complications because of pregnancies especially in the wild mice, the survey efforts concentrated only on male mice.

Table 18 gives a breakdown of the wild mice included in Table 17. These mice were captured in the summer of 1981 from corn cribs at 8 locations in southwestern Ontario. Each sample showed a significantly higher SCE value than C3H or C57BL mice maintained in the laboratory.

Wild mice showed an east to west gradient of SCE levels with mice from western sites generally having higher values. To determine if this pattern could be related to industrial and/or urban pollution, two correlations were calculated: i) between SCE values and the distance of the Windsor-Detroit complex (the largest industrial and urban center in the study area), and ii) between SCE values and the distance to the nearest industrial complex. A negative correlation was found in both cases ($r = -0.496$ and $r = -0.488$, respectively). Both correlation coefficient values are highly significant ($p < .0001$). Obviously, these correlations do not establish a definitive link between SCE values and industrial/urban emissions, they simply indicate the possibility of such a relationship.

Finally, inbred mice maintained in the corn filled enclosures on a farm east of a chemical manufacturing plant

Table 18: Mean SCE Values in Wild Mice Collected From Various Locations in Wouthwestern Ontario. (The Sites Run From East to West. Only Samples With Two or More Mice are Included).

| Location | No. of Mice Examined | Mean SCE/Cell ± SEM (per animal) | Distance (km) from Windsor/Detroit Complex | Distance (km) from Nearest Industrial Center* |
|---|---|---|---|---|
| Fingal | 2 | 5.37±0.13 | 154 | 86 |
| Wardsville | 4 | 5.36±0.53 | 115 | 62 |
| Ridgetown | 12 | 5.58±0.35 | 100 | 75 |
| Tilbury | 4 | 4.83±0.19 | 58 | 58 |
| Stoney Point | 8 | 6.38±0.13 | 48 | 48 |
| Harrow | 4 | 7.23±0.28 | 27 | 27 |
| Essex | 5 | 6.95±0.13 | 22 | 22 |
| McGregor | 8 | 6.27±0.31 | 19 | 19 |

*The closest industrial centers for all populations were either Windsor/Detroit or Sarnia, Ontario.

in Amherstburg, Ontario, just south of Windsor and on several farms east of Sarnia, Ontario, gave SCEs significantly higher ($p < .01$) than laboratory maintained inbred mice. In the Sarnia region (Table 19), a slight decrease in SCE values was observed as the distance from Sarnia increased. Although a larger sample size is required to determine whether this decrease is real, the pattern supports the findings from the corn crib populations that the mice closed to industrial sites showed a higher SCE value compared to those away from industrial centres.

## 4. CONCLUSION

A technique which gives consistently good preparations for the detection of sister chromatid exchanges (SCEs) in wild mice (Mus musculus) has been developed. This technique has permitted a comparison between inbred mice and mice from natural populations. Moreover, the differences in SCE values between laboratory maintained mice and wild mice, and between laboratory maintained mice and inbred mice housed in enclosures at various outdoor sites, together with the geographic patterns in SCE levels, suggest that this approach has potential as an early warning surveillance system for changes in the general levels of genotoxic agents in the environment.

Table 19: Mean SCEs in Inbred Male Mice Maintained in Outdoor Enclosures.

| Location | Distance from Industrial Center | Number of mice examined | SCE/Cell ± SEM. (per animal) |
|---|---|---|---|
| Sarnia I | 3 km | 7 | 5.21±0.13 |
| Sarnia II | 14 km | 3 | 4.90±0.10 |
| Sarnia III | 40 km | 8 | 4.48±0.23 |
| Amherstburg | 15 km | 4 | 5.42±0.17 |

# CHAPTER V

## GENERAL DISCUSSION

The continuous introduction of biologically hazardous chemicals to the ecosystem necessitates the development of short and long term testing procedures for monitoring the levels of genotoxic agents in the environment. Unfortunately, most of the current testing procedures do not have these features. The in vivo SCE method with serial injections of BUdR and FUdR does. Any test system designed to measure the levels of environmental genotoxic agents should possess the following qualities:

i) a high sensitivity to common genotoxic compounds;

ii) the capability of detecting pro-, ultimate-, and co-carcinogenic mutagens; and

iii) reliability, reproducibility, and economic feasibility.

In vivo testing appears to meet the above criteria.

The mechanisms of sister chromatid differentiation and SCE formation and the relationship of SCE to mutation have not been fully understood. It is believed that in uninemic chromatids when BUdR is substituted in only one helix of the DNA molecule, the chromatid takes a dark stain. When both strands in the chromatid show BUdR substitution, the chromatid appears lighter when stained with buffered Giemsa.

Zakharov and Egolina (1972) noted that after Giemsa

staining, the pale chromatid was usually longer than its sister. They postulated that protein synthesis that affected chromosomal condensation and spiralization was delayed by the substitution of thymidine by BUdR: David et al., (1974) concluded that proteins are more tightly bound to DNA containing BUdR than unsubstantiated DNA. Ikushima (1977) attributed the differential staining to a differential binding of proteins to the DNA of chromatin. Electron microscopic studies with Chinese hamster ovary cells have shown that the primary effect of the BUdR incorporation into chromosomes is exerted at the level of packing 25 nm fibres into larger chromosomal units. The bifilarly substituted chromatid is more open with looser gyres than is the unifilarly substituted chromatid (Wolff, 1977).

None of the hypotheses at the present time is satisfactory. However, it is known that the formation of SCE is linked to DNA synthesis and may involve breakage and reunion similar to meiotic recombination in order to conserve polarity in uninemic chromatids during the DNA synthetic phase (Taylor et al., 1957; Taylor, 1958). It is during this period, that the cell is also involved with the synthesis of RNA, histone, nonhistone proteins, many synthesis and repair enzymes, as well as the packing of the newly synthesized DNA into organized chromosomes. At

present, the temporal relationship of the above events with the formation of SCE is not known. However, recent studies (Carrano et al., 1978; Swenson et al., 1980) have demonstrated that there is a relationship between SCEs and point mutations but no clear relationship between SCEs and chromosomal aberrations has been established (Popescu et al., 1977). It has been reported that SCEs occur more frequently in euchromatic regions while chromatid aberrations occur more frequently in the heterochromatic regions (Wolff and Bodycote, 1975; Holmquist and Comings 1975; Ueda et al., 1976; Ikushima 1977; Schubert and Rieger, 1981).

Circumstantial evidence that SCEs and chromosomal aberrations originate from different chromatid lesions is also derived from a number of human autosomal chromosome fragility diseases such as Fanconi's anaemia, Bloom's syndrome, ataxia-telangiectasis and xeroderma pigmentosa (German, 1972). A high SCE frequency was demonstrated in metaphases of patients with Bloom's syndrome (Chaganti et al., 1974). Similar evidence has also been obtained from other experimental studies (Anderson et al., 1981).

When root tips of Vicia faba were treated with increased concentrations of the antibiotic, streptonigrin, the frequencies of SCEs and chromosomal aberrations increased proportionately (Anderson et al., 1981). Other studies with X or $\gamma$-rays have shown that an increase in

SCEs occurred when doses produced high frequencies of chromosomal aberrations (Gatti and Olivieri, 1973; Perry and Evans, 1975). All the above agents (streptonigrin, X/or $\gamma$-rays) are known as S-independent inducers of SCE (Anderson et al., 1981). These observations also suggest that different DNA lesions are involved in producing chromosomal aberrations, and SCEs.

The present method of analysing SCEs in bone marrow cells of natural populations of wild and inbred mice exposed to outdoor atmospheric pollutants appears to be sensitive, reproducible and practical in the detection of environmental genotoxicity. The technique involves 9 serial injections of 40 ug/g of b.w. of BUdR, 2 ug/g of b.w. of FUdR and 5 ug/g of b.w. of colchicine on the 27th hr for three hours. The cells were collected and treated with 0.075M KCl at 37°C and fixed in four changes of methanol:glacial acetic acid (3:1). The slides were incubated in 1M phosphate buffer for 15 min at 89°C. The technique has given good and consistent results and has also provided permanent preparations. The 27-30 hr time schedule was preferred because it gave a minimal number of aberrations compared with the earlier time schedules and is applicable to the wild as well as inbred strains of mice. Each time schedule produced a characteristic SCE value.

The baseline SCEs are dependent on the system used and the procedure followed (Carrano et al., 1980) but

appear little affected by the genotypes of the mice used. The similar base values observed in the present study in mice housed under laboratory control conditions are similar to those reported by other workers using similar approaches (Bauknecht et al., 1977; Vogel and Bauknecht, 1976; Dragani et al., 1981). When the mode of administration of BUdR is intravenous, subcutaneous or subcutaneously implanted or BUdR activated to charcoal, the baseline count of SCEs varies (Kram and Schneider, 1978; Ramirez, 1980). The baseline value may also vary with the species used but only marginally in different strains (Roszinsky-Kocher and Rohrborn, 1979; Vogel and Bauknecht 1976). Other conditions which may affect the levels of differentiated metaphases include: photolysis of BUdR, room temperature during the injection period and the time of sacrificing of the animals. The pH and molarity of the solution used in making the BUdR and FUdR may have some effect on the percentage of differentiation metaphase (Kato, 1974c and Burkholder 1978) but do not appear to affect the SCE levels. Similarly, individuals in a given treatment group may vary in the percentage of differentiated metaphases but SCE frequencies remained very similar. A preliminary study shows that the induction of SCE due to MMC may depend on the exposure time. This aspect of the testing system will be examined in future to improve the sensitivity of the technique. Present studies

indicate that males are better experimental animals for SCE evaluation than females and also, inbred strains such as C3H and C57BL are suitable controls.

Bone marrow cells appeared as excellent tissues for SCE analysis because they are easily obtained and show a high mitotic index. Allen et al. (1978) and Pallitti et al. (1982) have shown that the baseline SCE values in bone marrow cells are very similar to the SCE values observed in spleen and thymus cells. Therefore, bone marrow cells appear to give SCE values that are consistent with somatic cells.

The fact that the wild mice maintained in the laboratory for six months or more have similar base counts to those observed in inbred strains suggests that both are suitable as controls. Stetka et al. (1978) investigated the decrease in SCE frequencies over time in experimental animals and found that the repeated administration of low doses of benzo(a)pyrene, methylcholanthrene and MMC resulted in an increased frequency of SCEs that persisted for several months after the final injections. This finding suggests that the long term exposure to certain SCE inducing chemicals may also be monitored with the SCE test.

The major contributions of the present study include:

i) The development of a working in vivo SCE system applicable to inbred and wild mice for general measuring of DNA damaging effects of environmental

agents.

ii) The establishment of baseline SCEs in various strains of mice and in wild mice maintained in the laboratory.

iii) The dose effects of BUdR, FUdR and Mitomycin-C on cell survival, percentage of differentiated metaphases and incidence of SCEs.

iv) The effect of various injection schedules on the percentage of differentiated metaphases, SCEs and chromosomal aberrations.

v) The correlation between SCE values in mice and the exposure of these animals to industrial pollution.

# CHAPTER VI

## GENERAL CONCLUSIONS

Based on the results and discussion presented in this dissertation, *in vivo* SCE analysis, using murine bone marrow cells, is a viable whole animal test system for monitoring general levels of genotoxicity of chemicals dumpsites, industrial atmospheric pollutants and contaminated water. Furthermore, this approach may be used for *in vivo* evaluations of a number of suspected chemical mutagens including agriculturally used pesticides.

# APPENDIX I

## Formulae for Solutions

A. Hanks Balanced Salt Solution (HBSS)

Solution a:

| | |
|---|---|
| NaCl | 4000 mg |
| KCl | 200 mg |
| Glucose | 500 mg |
| Dist $H_2O$ | 200 ml |

Solution b:

| | |
|---|---|
| $KH_2PO_4$ | 30 mg |
| $Na_2HPO_4.7H_2O$ | 45 mg |
| $NaHCO_3$ | 175 mg |
| Dist $H_2O$ | 300 ml |

Solution b was mixed with solution a and the mixture was autoclave sterilized before use.

B. Dulbecco's Phosphate Buffer

| | |
|---|---|
| KCl | 20 mg |
| $KH_2PO_4$ | 20 mg |
| NaCl | 800 mg |
| $Na_2HPO_4.7H_2O$ | 216 mg |
| Dist $H_2O$ | 100 ml |

The solution was autoclave sterilized before use.

C. Sörensen Buffer

| | |
|---|---|
| $KH_2PO_4$ | 663 mg |
| $Na_2HPO_4.7H_2O$ | 256 mg |
| Dist $H_2O$ to | 100 ml |

D. Schiff's Reagent

| | |
|---|---|
| Basic fuchsin | 1 g |
| Dist $H_2O$ | 200 ml |
| 1N HCl | 20 ml |
| Potassium metabisulphite | 1 g |
| Activated charcoal | 2 g |

The staining procedure followed as per the instructions of Haboswky (1968).

# APPENDIX II

Table 20. Mean SCE values in male wild mice maintained in the laboratory over a period of 6-9 months.

| Group | Location | Animal No. | Body wt. | SCE±SEM |
|---|---|---|---|---|
| I | Laboratory | C3H/J (Control) | 23 g | 3.80±0.22 |
| | Martin | 81-681 | 21 g | 4.10±0.33 |
| | Martin | 81-688 | 20 g | 3.86±0.24 |
| | Martin | 81-700 | 17 g | 3.60±0.28 |
| | Martin | 81-704 | 22 g | 3.80±0.20 |
| II | Laboratory | C3H/J (Control) | 20 g | 3.90±0.33 |
| | Laramie | 81-817 | 22 g | 3.45±0.28 |
| | Laramie | 81-818 | 24 g | 4.17±0.40 |
| | Laramie | 81-841 | 20 g | 3.60±0.25 |
| | Laramie | 81-846 | 22 g | 3.89±0.27 |
| III | Laboratory | C3H/J (Control) | 24 g | 3.00±0.20 |
| | Houle | 80-637 | 18 g | 2.87±0.30 |
| | Houle | 80-650 | 20 g | 2.85±0.32 |
| | Houle | 80-601 | 23 g | 3.01±0.26 |
| | Laramie | 80-232 | 22 g | 2.75±0.26 |
| | Schaffer | (79-851 79-855) | 18 g | 3.00±0.12 |

Group I and II maintained for six months and group III for a period of nine months.

Table 21: SCE values in individual wild mice.

| Date of Collection. | | | | | | |
|---|---|---|---|---|---|---|
| Yr. | Month | Day | Location | Animal No. | Body wt. | SCE±SEM |
| 81 | 6 | 24 | Lab maintained | C57BL/6J (Control) | 30 g | 3.90±0.40 |
| 81 | 6 | 24 | Houle | 81-644 | 24 g | 5.80±0.30 |
| 81 | 6 | 24 | Houle | 81-647 | 24 g | 5.50±0.29 |
| 81 | 6 | 24 | Houle | 81-653 | 20 g | 6.10±0.31 |
| 81 | 6 | 24 | Houle | 81-667 | 20 g | 5.50±0.32 |
| 81 | 6 | 24 | Houle | 81-673 | 23 g | 6.25±0.33 |
| 81 | 6 | 24 | Houle | 81-663 | 21 g | 5.80±0.24 |
| 81 | 6 | 27 | Lab maintained | C57BL/6J (Control) | 23 g | 3.75±0.25 |
| 81 | 6 | 27 | Carmichael | 81-567 | 21 g | 5.24±0.38 |
| 81 | 6 | 27 | Carmichael | 81-583 | 19 g | 5.50±0.26 |
| 81 | 7 | 15 | Lab maintained | C57BL/6J (Control) | 28 g | 3.30±0.36 |
| 81 | 7 | 15 | Wardsville | 81-723 | 16 g | 6.06±0.31 |
| 81 | 7 | 15 | Wardsville | 81-724 | 18 g | 6.43±0.33 |
| 81 | 7 | 15 | Wardsville | 81-727 | 13 g | poorly diff. metaphases |
| 81 | 7 | 15 | Wardsville | 81-732 | 18 g | 4.80±0.22 |
| 81 | 7 | 15 | Wardsville | 81-733 | 22 g | 4.15±0.40 |
| 81 | 7 | 15 | Wardsville | 81-746 | 15 g | poorly diff. metaphases |

Table 21 (contd)

| Date of Collection Yr. | Month | Day | Location | Animal No. | Body wt. | SCE±SEM |
|---|---|---|---|---|---|---|
| 81 | 7 | 16 | Lab maintained | C57BL/6J (Control) | 33 g | 3.50±0.31 |
| 81 | 7 | 16 | Gagnier (Stoney Point) | 81-713 | 23 g | 7.43±0.28 |
| 81 | 7 | 16 | Quinton (Stoney Point) | 81-710 | 20 g | 8.65±0.45 |
| 81 | 7 | 16 | Lab maintained | C57BL/6J (Control) | 20 g | 3.50±0.31 |
| 81 | 7 | 16 | Ridgetown | 81-1025 | 18 g | died during BUdR/FUdR inj. |
| 81 | 7 | 16 | Ridgetown | 81-1028 | 22 g | 6.00±0.42 |
| 81 | 7 | 16 | Ridgetown | 81-1032 | 20 g | 5.60±0.40 |
| 81 | 7 | 16 | Ridgetown | Not recorded | 20 g | 5.60±0.40 |
| 81 | 7 | 16 | Ridgetown | Not recorded | 22 g | 6.45±0.42 |
| 81 | 7 | 16 | Lab maintained | C57BL/6J | 33 g | 3.50±0.31 |
| 81 | 7 | 16 | Laramie | 81-1051 | 18 g | 5.09±0.30 |
| 81 | 7 | 16 | Laramie | 81-1054 | 21 g | 6.75±0.32 |
| 81 | 7 | 16 | Laramie | Not recorded | 21 g | 5.40±0.29 |
| 81 | 7 | 16 | Laramie | Not recorded | 22 g | 6.50±0.30 |
| 81 | 8 | 1 | Lab maintained | C3H/J | 23 g | 3.30±0.29 |
| 81 | 8 | 1 | Ridgetown | 81-751 | 21 g | 5.95±0.45 |
| 81 | 8 | 1 | Ridgetown | 81-763 | 20 g | 6.00±0.20 |

Table 21 (contd)

| Date of Collection Yr. | Month | Day | Location | Animal No. | Body wt. | SCE±SEM |
|---|---|---|---|---|---|---|
| 81 | 8 | 1 | Ridgetown | 81-765 | 21 g | 3.76±0.29 |
| 81 | 8 | 1 | Ridgetown | 81-774 | 23 g | 5.70±0.41 |
| 81 | 8 | 1 | Ridgetown | 81-776 | 22 g | 3.90±0.17 |
| 81 | 8 | 1 | Ridgetown | 81-778 | 21 g | 4.73±0.49 |
| 81 | 8 | 7 | Lab maintained | C3H/J (Control) | 20 g | 3.30±0.30 |
| 81 | 8 | 7 | Huron Line (Windsor) | 81-783 | 24 g | 6.20±0.48 |
| 81 | 8 | 7 | Huron Line (Windsor) | 81-784 | 13 g | very few poorly diff. metaphases |
| 81 | 8 | 7 | Morpeth | 81-786 | 16 g | 5.50±0.34 |
| 81 | 8 | 7 | Morpeth | 81-785 | 20 g | 6.34±0.32 |
| 81 | 8 | 7 | Paincourt | 81-779 | 24 g | 6.13±0.35 |
| 81 | 8 | 28 | Lab maintained | C3H/J (Control) | 27 g | 3.30±0.20 |
| 81 | 8 | 28 | Essex | 81-959 | 16 g | 6.60±0.30 |
| 81 | 8 | 28 | Essex | 81-964 | 18 g | 7.27±0.34 |
| 81 | 8 | 28 | Essex | 81-969 | 19 g | 7.00±0.30 |
| 81 | 8 | 28 | Essex | 81-971 | 16 g | 7.20±0.32 |
| 81 | 8 | 28 | Essex | 81-972 | 21 g | 6.70±0.47 |
| 81 | 8 | 13 | Lab maintained | C57BL/6J | 27 g | 3.30±0.28 |
| 81 | 8 | 13 | Laramie | 81-820 | 18 g | 6.80±0.38 |

Table 21 (contd

| Date of Collection Yr. | Month | Day | Location | Animal No. | Body wt. | SCE±SEM |
|---|---|---|---|---|---|---|
| 81 | 8 | 13 | Laramie | 81-843 | 22 g | 7.00±0.34 |
| 81 | 8 | 13 | Laramie | 81-850 | 17 g | 5.60±0.26 |
| 81 | 8 | 13 | Laramie | 81-851 | 18 g | 6.40±0.39 |
| 81 | 8 | 17 | Lab. maintained | C3H/J (Control) | 20 g | 3.75±0.20 |
| 81 | 8 | 17 | Trudell | 81-855 | 17 g | 4.57±0.34 |
| 81 | 8 | 17 | Trudell | 81-856 | 19 g | 5.30±0.38 |
| 81 | 8 | 17 | Trudell | 81-860 | 20 g | 4.81±0.47 |
| 81 | 8 | 17 | Trudell | 81-863 | 17 g | 4.57±0.30 |
| 81 | 9 | 1 | Lab maintained | $F_1$ (C3H♂x C57BL♀) | 17 g | 3.90±0.29 |
| 81 | 9 | 1 | Fox | 81-1100 | 16 g | 7.80±0.50 |
| 81 | 9 | 1 | Fox | 81-1108 | 20 g | 6.95±0.33 |
| 81 | 9 | 1 | Fox | 81-1110 | 20 g | 7.50±0.34 |
| 81 | 9 | 1 | Fox | 81-1114 | 16 g | 6.59±0.32 |

Table 22: Mean SCE values in inbred mice maintained in enclosures in the Sarnia area.

| Locations | Strains | Sex | Body wt. | Duration of time on the farm | SCE±SEM |
|---|---|---|---|---|---|
| I (Archer Farm) | C3H/J (Control) | male | 20 g | | 3.45 |
| | C3H/J | male | 25 g | 3 weeks (12/8/81-12/29/81) | 5.84±0.32 |
| | C3H/J | male | 25 g | " | 5.00±0.32 |
| | C3H/J | male | 28 g | 10 weeks (10/9/81-12/29/81) | 5.29±0.25 |
| | C3H/J | male | 22 g | " | 4.80±0.29 |
| | C3H/J | male | 24 g | " | 5.39±0.39 |
| | C3H/J | male | 22 | " | 5.00±0.20 |
| | C57BL/6J | male | 24 | " | 5.17±0.17 |
| IIa (Elliot Farm) | C3H/J | male | 20 g | 7 weeks (10/20/81-12/14/81) | 5.44±0.31 |
| IIb (Buhlman Farm) | C3H/J | male | 24 g | 3 weeks (12/8/81-12/9/81) | 4.75±0.30 |
| | C57BL/6J | male | 24 g | 7 weeks (10/20/81-12/14/81) | 4.52±0.44 |
| III (Leeson Farm) | C3H/J | male | 25 g | 3 weeks | 3.50±0.30 |
| | C3H/J | male | 24 g | 7 weeks | 3.32±0.17 |
| | C3H/J | male | 25 g | 10 weeks | 3.40±0.26 |
| | C3H/J | male | 24 | " | 4.32±0.29 |
| | C3H/J | male | 20 g | " | 3.92±0.25 |
| | C3H/J | male | 22 g | " | 5.80±0.30 |
| | C3H/J | male | 26 g | " | 5.90±0.39 |
| | C3H/J | male | 25 g | " | 5.25±0.30 |
| | C3H/J | female | 26 g | 3 weeks (12/5/81-12/29/81) | 5.00±0.33 |

Table 22 (contd)

| Locations | Strains | Sex | Body wt. | Duration of time on the farm | SCE±SEM |
|---|---|---|---|---|---|
| (Leeson Farm) | C3H/J | female | 25 g | 7 weeks (10/20/81-12/14/81) | 5.43±0.24 |
| | C3H/J | female | 25 g | " | 4.90±0.25 |
| | C3H/J | female | 24 g | 12 weeks (9/29/81-12/29/81) | 5.80±0.38 |
| | C3H/J | female | 25 g | " | 5.50±0.34 |

Location I is 3.3 km from Polysar Chemical Industries
Location IIa is 10 km " " " "
Location IIb is 18 km " " " "
Location III is 40 km " " " "

Table 23: Frequency of SCE in inbred mice maintained in Martin's farm

| | Animal No. | sex | Body wt. | No. of weeks on the farm | SCE±SEM |
|---|---|---|---|---|---|
| I | C3H/J (Control) | female | 24 g | | 3.75±0.55 |
| | Martin wild (80-478) | male | 20 g | 5 | 4.56±0.26 |
| | C57BL/J | female | 25 g | 5 | 6.18±0.45 |
| | C57BL/6J | female | 25 g | 5 | 6.20±0.39 |
| | Martin wild (80-470) | male | 20 g | 5 | 5.15±0.46 |
| II | C57BL/6J (Control) | male | 24 g | | 3.70±0.32 |
| | C57BL/6J | male | 22 g | 12 | 5.82±0.37 |
| | C3H/J | female | 24 g | 12 | 7.22±0.32 |
| | C3H/J | female | 24 g | 12 | 5.65±0.43 |
| III | C3H/J (Control) | male | 20 g | | 3.60±0.39 |
| | C3H/J | male | 22 g | 14 (9/29/81-1/11/82) | 5.30±0.22 |
| | C3H/J | male | 20 g | " | 5.54±0.26 |
| | C57BL/6J | female | 24 g | " | 6.15±0.24 |
| | C57BL/6J | female | 25 g | " | 6.35±0.24 |

The second group of mice were given BUdR/FUdR injections one hour after they were brought to the laboratory. Other field mice were treated within 24 hours of their capture.

Table 24: t-test Comparisons of SCE Means

| Comparisons | p |
|---|---|
| C3H males 3.42* (19) vs C57BL males 3.62(17) | $<.05$ |
| C3H males 3.42(19) vs C3H females 5.09(4) | $<.01$ |
| C3H males 3.42(19) vs $F_1$ males (C3H♂ x C57BL♀) 4.13(4) | $<0.05$ |
| C3H males 3.42(19) vs $F_1$ females (C3H♂ x C57BL♀) 6.16(2) | $<0.01$ |
| C3H males 3.42(19) vs DBA males 3.97(3) | $<0.05$ |
| C3H males 3.42(19) vs cornfed C3H males 3,81(3) | $<0.05$ |
| C3H males 3.42(19) vs laboratory maintained wild males 3.46(13) | |
| C3H males 3.42(19) vs freshly caught wild males 6.02(49) | $<.01$ |
| C3H males 3.42(19) vs C3H males from Sarnia 4.83(18) | $<.01$ |
| C3H males 3.42(19) vs C3H males from Martin's 5.42(3) | $<.01$ |
| C57BL males 3.62(17) vs C57BL females 5.71(4) | $<.01$ |
| C57BL females 5.71(4) vs C3H females 5.09(4) | $<.01$ |
| C57BL males 3.62(17) vs $F_1$ males (C3H♂ x C57BL♀) 4.13(4) | $<.05$ |
| C57BL males 3.62(17) vs DBA males 3.97(3) | $<.05$ |
| C57BL males 3.62(17) vs freshly caught wild males 6.02(49) | $<.01$ |
| C3H males from Sarnia I 5.21(7) vs C3H males from Sarnia III 4.88(8) | $<0.20$ |
| C3H males from Sarnia pooled 4.83(18) vs C3H males from Martin's 5.42 | |
| C3H males from Sarnia and Martin's 4.44(21) vs freshly caught wild males 6.02(49) | $<0.01$ |

*Mean SCE value for the group and the figure in parenthesis is the number of individuals analysed for the SCE count

BIBLIOGRAPHY

Abe, S. and Sasaki, M. (1977a) Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. Journal Nat. Inst. 58:1635-1641.

Abe, S. and Sasaki, M. (1977b) Studies in chromosome aberrations and sister chromatid exchanges induced by chemicals. Proc. Jap. Acad. 53:46-49.

Aebersold, P.M. (1976) Mutagenic mechanism of 5-bromodeoxyuridine in Chinese hamster cells. Mutation Res. 36:357-362.

Aebersold, P.M. (1979) Mutation by 5-fluorodeoxyuridine in synchronous Chinese hamster cells. Cancer Res. 39:808-810.

Allen, J.W. and Latt, S.A. (1976a) Analysis of sister chromatid exchange formation in vivo in mouse spermatogonia as a new test system for environmental mutagens. Nature (London) 260:449-451.

Allen, J.W. and Latt, S.A. (1976b) In vivo BUdR-33258 Hoechst analysis of DNA replication kinetics and sister chromatid exchange formations in mouse somatic and meiotic cells. Chromosoma 58:325-340.

Allen, J.W. and Latt, S.A. (1977) Bromodeoxyuridine tablets methodology in in vivo studies. Somatic Cell Genetics 4: 393-405.

Allen, J.W., Schuler, C.F., and Latt, S.A. (1977) A simplified technique for in vivo analysis of sister chromatid exchanges using 5-bromodeoxyuridine tablets. Cytogenetics Cell Genetics (Basel) 18:231-237.

Allen, J.W., Schuler, C.F., and Latt, S.A. (1978) BRdU tablet methodology for in vivo studies of DNA synthesis. Somatic Cell Genetics 4:393-405.

Ames, B.N. (1979) Identifying environmental chemicals causing mutations and cancers. Science 204:587-592.

Ames, B.N., Durston, W.E., Yamasaki, E., and Lee, F.D. (1973) Carcinogens and mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. (USA) 70:2281.

Ames, B.N., McCann, J., and Yamasaki, E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test. Mutation Res. 31: 347-364.

Anderson, D. (1979) Short term tests for detecting chemical mutagens and recommendations for their use in monitoring exposed populations. Genetic Damage in Man Caused by Environmental Agents, edited by Kare Berg, New York, Academic Press, 1979, pp. 383-404.

Anderson, H.C., Kihlman, B.A. and Pallitti, F. (1981) Production of sister chromatid exchanges by x-rays under aerobic and anaerobic conditions. Hereditas 94:41-44.

Barrett, H.W. and West, R.A. (1956) Rapid dehalogenation of BUdR in the liver. J. Am. Sc. 78:1612-1615.

Bartsch, H. and Montesano, R. (1975) Mutagenic and carcinogenic effects of vinyl chloride. Mutation Res. 32:93-114.

Bateman, A.J. and Epstein, S. (1971) Chemical Mutagenesis, Vol. II. edited by A. Hollander, New York, Plenum Press, p. 541.

Bauknecht, T.H., Vogel, W., Bayer, U. and Wild, D. (1977) Comparative in vivo mutagenicity testing by SCE and micronucleus in mouse bone marrow. Human Genetics 35:299-307.

Bayer, U., and Bauknecht, T.H. (1977) The dose dependence of sister chromatid exchanges in the in vivo bone marrow test with Chinese hamster cells induced by 3-hydrocarbons. Experientia (Basel) 35:25.

Beckman, L. and Nordström, M. (1976) Population studies in northern Sweden. VIII. Frequencies of congenital malformations by regions, times, sex, and maternal age. Hereditas 84:35.

Beek, B. and Obe, G. (1975) The human leukocyte test systems. VI. The use of sister chromatid exchanges as a possible indicator for mutagenic activities. Human Genetik 29(2): 127-134.

Bender, M.A., Griggs, H.G. and Walker, P.L. (1974) Mechanism of chromosomal aberration production. I. Aberrations induced by ultraviolet light. Mutation Res. 20: 387-402.

Benditt, E.P. (1977) The origins of atherosclerosis. Sc. Am. 236(2):74-85.

Bigger, C.A.H., Tomuszewski, J.E., and Dipple, A. (1978) Differences between products of binding of 7,12 dimethylbenzanthracene to DNA in mouse skin and in rat liver microsomal system. Biochem. Biophys. Res. Comm. 80:229-235.

Bloom, S.E. and Hsu, T.C. (1975) Differential fluorescence of sister chromatids in chicken embryos exposed to 5-bromodeoxyuridine. Chromosoma 51:261-267.

Blot, W.T., Brinton, L.A., Fraument, J.F. and Stone, B.J. (1977) Cancer mortality in U.S. counties with petroleum industries. Science 198:(4312):51-53.

Bostock, C.J. and Sumner, A.T. (1978) The Eukaryotic Chromosomes. North Holland Publishing Co. Amsterdam, New York, Oxford, pp. 437-445.

Bridges, B.A. (1972) Simple bacteria systems for detecting mutagenic agents. Lab. Practice 21:413-416.

Brinkley, B.R. and Humphrey, R.M., (1969) Evidence for subchromatid organization in marsupial chromosomes. J. Cell Biology 42:827.

Brøgger, A. (1979) Chromosome damage in human mitotic cells after in vivo and in vitro exposure to mutagens in Genetic Damage in Man Caused by Environmental Agents. Edited by K. Berg, New York, Academic Press, 1979, pp. 87-97.

Brown, R.L. and Crossen, P.E. (1976) Increased incidence of sister chromatid exchanges in Rauscher leukemia virus infected mouse embryo fibroblasts. Exp. Cell Res. 103:418-420.

Burkholder, G.D. (1978) Reciprocal giemsa staining of late DNA replicating regions produced by low and high pH sodium phosphate. Exp. Cell. Res. 111:489-492.

Burnet, F.M. (1974) Intrinsic Mutagenesis: A Genetic Approach to Aging. Medical and Technical Publishing. Lancaster, England.

Carrano, A.V., Thompson, L.H., Lindl, P.A. and Minkler, J.L. (1978) Sister chromatid exchange as an indicator of mutagenesis. Nature (London) 271:551-553.

Carrano, A.V., Minkler, J.L., Stetka, D.G. and Moore, D.H. II. (1980) Variation in the baseline sister chromatid exchange frequency in human lymphocytes. Environmental Mutagenesis 2(3):325.

Chaganti, R.S.K., Schonberg, S. and German, J. (1974) A many fold increase in sister chromatid exchange in Bloom's Syndrome lymphocytes. Proc. Nat. Acad. Sci. (USA) 71(11): 4508-4512.

Chrisp, C.E. and Fisher, G.L. (1980) Mutagenicity of airborne particles. Mut. Res. 76:143-144.

Choudhuri, N.K., Montag, B.J. and Heidelberger, C. (1958) Studies of fluorinated pyrimidines. III. The metabolism of 5-fluorouracil-2-$C^{14}$- and 5 fluoroorotic-2-$C^{14}$ acid in vivo. Can. Res. 18:318-328.

Cohen, M.M. and Shaw, M.W. (1964) Effects of mitomycin-C on human chromosomes. J. Cell Biology 23:386-395.

Crossen, P.E., Morgan, W.F., Horan, J.J., Stewart, J. (1978) Cytogenetic studies of pesticides and herbicide sprayers. Newzealand Med. J. 88:192-195.

Csukas, I., Gungl, E., Fedorcsak, I., Vida, G., Antoni, F., Turtoczsky, I. and Solymosy, F. (1979) Urethane and hydroxyurethane induced sister chromatid exchanges in cultured human lymphocytes. Mut. Res. 67:315-319.

David, J., Gordon, J.S., Rutter, W.J. (1974) Increased thermal stability of chromatin containing 5-bromodeoxyuridine substituted DNA. Proc. Nat. Acad. Sci. (USA) 71:2808-2812

DeCassia, S.R., Geslie, C.C., da Cruz, G.C.C., Saliba, F., Becak, W.W. (1981) Cytogenetic effects of an organophosphate pesticide on cattle and their progeny. Rev. Brazil. Genet. 4(1):55-63.

DeRatt, W.K. (1978) Induction of sister chromatid exchanges by styrene and its presumed metabolite styrene oxide in the presence of rat liver homogenate. Chem. Biol. Interact. 20:163-170.

Dewey, W.C. and Humphrey, R.M. (1965) Increase in radiosensitivity to ionizing radiation related to replacement of thymidine in mammalian cells with 5-bromodeoxyuridine. Radiat. Res. 26:538-553.

Doll, R. (1977) Strategy for detection of cancer hazards to man. Nature (London) 265-589.

Doll, R., Matthews, J.D. and Morgan, L.G. (1977) Cancer of the lung and nasal sinuses in nickel workers: a reassessment of the period of risk. Br. J. Industrial Med. 34:102-109.

Doll, R., Morgan, L.G. and Speiger, F.E. (1970) Cancer of the lung and nasal sinuses in nickel workers. Br. J. Canc. 24:623-632.

Douglas, G.R., Bell, R.D.I., Grant, C.E., Wytsma, J.M. and Bora, K.C. (1980) Effect of lead chromate on chromosomal aberrations, sister chromatid exchange and DNA damage in mammalian cells in vitro. Mut. Res. 77(2):. 157-164.

Dragani, T.A., Zurino, A. and Sozzi, G. (1981) Differences in sister chromatid exchanges induction in vivo by cyclophosphamide in murine strains. Carcinogenesis 2(3):219-222.

Ehling, U.H. (1975) Dominante lethalmutationen bei mausen. G.S.F. Bericht B 745:8.

Eigesti, O.J. and Dustin, P. (1955) Colchicine in agriculture, medicine, biology and chemistry. Iowa State College Press, Iowa.

Evans, H.J. (1962) Chromosome aberrations induced by ionizing radiation. Int. Rev. Cyt. 13:221-321.

Evans, H.J. (1977) Molecular mechanisms in the induction of chromosomal aberrations in D. Scott, B.A. Bridges and F.H. Sobels (eds) Progress in Genetic Toxicology, Elsevier/North-Holland, Amsterdam. pp. 55-74.

Evans, J. and O'Riordan, M.L. (1975) Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutation Res. 31:135-148.

Evans, H.J. and Scott, D. (1969) The induction of chromosome aberration by nitrogen mustard and its dependence on DNA synthesis. Proc. Royal Soc. (London) 173:491-512.

Fahrig, R. (1975) A mammalian spot test induction of genetic alteration in pigment cells of mouse embryos with x-rays and chemical mutation. Mol. Genetics 138: 309-314.

Fremuth, F., Barta, I., Sadilkova, M. (1976) Radiation injury of chromosomes during the cell cycle in vivo. Stud. Biophy. 54(3):169-185.

Gabridge, M.G. and Legator, M.S. (1969) A host mediated microbial assay for the detection of mutagenic compounds. Proc. Soc. Exp. Biol. Med. 130:831-834.

Galloway, S.M. (1977) Ataxia telangiectasis: the effects of chemical mutagens and x-rays on SCE in blood lymphocytes. Mutation Res. 45:343-349.

Gatti, M. and Olivieri, G. (1973) The effects of x-rays on labelling patterns of $M_1$ and $M_2$ chromosomes in Chinese hamster cells. Mutation Res. 17:101-112.

Generoso, W.M., Cain, K.T. and Huff, S.W. (1978) Chromosomal aberration effects of benzo(a)pyrene in male and female germ cells of mice. Mutation Res. 53:126.

German, J. (1972) Genes which increase chromosomal instability in somatic cells and predispose to cancer. Prog. Med. Genet. 8:61-101.

German, J., Schonberg, S., Loue, E., and Chaganti, R.S.K. (1977) Bloom's Syndrome. IV. Sister chromatid exchanges in lymphocytes. Amer. J. Hum. Genet. 29:248-255.

Gibson, D.A. and Prescott, D.M. (1972) Induction of sister chromatid exchanges in chromosomes of rat kangaroo cells by tritium incorporated into DNA. Exp. Cell. Res. 74:397-402.

Giebhart, E. and Kappauf, H. (1978) Bleomycin and sister chromatid exchanges in human lymphocyte chromosomes. Mut. Res. 58:121-124.

Green, T. and Hathaway, D.E. (1975) The biological fate in rats of vinyl chloride in relation to its oncogenicity. Chem. Biol. Interact. 11:545-562.

Habowsky, J.E.H. (1968) Manual on Principle Methods in Cytology and Histology, Department of Biology, University of Windsor, Windsor, ON. pp. 37.

Haugh, T.H. (1982a) Biological markers for chemical exposure. Science 215(5):643-647.

Haugh, T.H. (1982b) Just how hazardous are dumps? Science 214(29):490-493.

Hartmann, K.U. and Heidelberger, C. (1961) Studies on fluorinated pyrimidines. Fluorinated pyrimidines. XIII. Inhibition of thymidylate synthetase. J. Biol. Chem. 263: 3006-3013.

Heddle, J.A. (1973) A rapid in vivo test for chemical damage. Mutation Res. 18:187-190.

Hiatt, H.H., Watson, J.D., Winston, J.A. (1977) Origin of Human Cancer, Cold Spring Habor Laboratory, Coldspring Harbor, N.Y.

Hollstein, M.A. and McCann, J. (1979) Short term tests for carcinogens and mutagen. Mutation Res. 65:133-226.

Holmquist, G.P. and Comings, D.E. (1975) Sister chromatid exchange and chromosome organization based on bromodeoxyuridine Giemsa C-banding technique. Chromosoma (Berl) 52 (3):245-259.

Hsu, T.C. and Somers, C.E. (1961) Effect of 5-bromodeoxyuridine on mammalian chromosomes. Proc. Natl. Acad. Sci. (Wash. USA) 47:396-403.

Heidelberger, C. (1965) Fluorinated pyrimidines. Proc. Nucleic Acid Res. 4:1-50.

Huang, C.G. (1967) Induction of a high incidence of damage to the x-chromosomes of Rattus (Mastomys) natalensis by analogues, viruses and carcinogens. Chromosoma 23:162-179.

Hughes, A. (1952) The Mitotic Cell, New York, Academic Press.

Huttner, K.M. and Ruddle, F.H. (1976) Study of mitomycin-C induced chromosomal exchange. Chromosoma 56:1-13.

Iijiama, T. and Hagiwara, A. (1960) Mutagenic action of mitomycin-C on Escherichia coli. Nature (London) 185: 395-396.

Ikushima, T. (1977) Role of sister chromatid exchange in chromosome aberration formation. Nature (London) 268: 235-236.

Ishii, Y. (1981) Nature of the mitomycin-C induced lesion causing sister chromatid exchanges. Mutation Res. 91: 51-551.

Ishii, Y. and Bender, M. (1978) Factors influencing the frequency of mitomycin-C induced sister chromatid exchanges in 5-bromodeoxyuridine substituted human lymphocytes in culture. Mutation Res. 51:411-418.

Jenssen, D., Ramel, C., and Gothe, R. (1974) The induction of micronuclei by frameshift mutagens at the time of nucleus expulsion in mouse erythroblasts. Mutation Res. 26:553-555.

Jose, J.G. (1979) Photomutagenesis by chlorinated phenothiazine tranquillizers. Proc. Nat. Acad. Sci. USA 76: 409.

Kapp, R.W., Picciano, D.J., Jacobson, C.B. (1979) Y-chromosomal nondysjunction in dibromochloropropane exposed workers. Mut. Res. 64:47-51.

Kato, H. (1973) Induction of sister chromatid exchanges by u.v. light and its inhibition by caffeine. Exp. Cell Res. 82:382-390.

Kato, H. (1974a) Induction of sister chromatid exchanges by chemical mutagens and its possible relevance to DNA repair. Exp. Cell Res. 85:239-247.

Kato, H. (1974b) Possible role of DNA synthesis in function of sister chromatid exchanges. Nature (London) 252:739-741.

Kato, H. (1974c) Spontaneous sister chromatid exchanges detected by the BUdR-labeling method. Nature (London) 251: 70-72.

Kato, H. (1977a) Mechanisms for sister chromatid exchanges and their relation to the production of chromosomal aberrations. Chromosoma 59:179-191.

Kato, H. (1977b) Spontaneous and induced sister chromatid exchanges as revealed by the BUdR-labeling method. Int. Rev. Cytol. 37:55-95.

Kato, H. and Sandberg, A. (1979) Effects of herpes simplex virus on sister chromatid exchange and chromosome abnormalities in human diploid fibroblasts. Exp. Cell Res. 109: 423-427.

Kato, H. and Shimada, H. (1975) Sister chromatid exchanges induced by mitomycin-C: a new method of detecting DNA damage at the chromosomal level. Mutation Res. 28:459-464.

Kihlman, B.A. (1975) Sister chromatid exchanges in Vicia faba II effects of thiotepa, caffeine and 8-ethoxy caffeine in the frequency of SCEs. Chromosoma 51: 11-18.

Korenberg, J.R. and Freedlender, E.F. (1974) Giemsa technique for the detection of sister chromatid exchanges, Chromosoma 48:355-360.

Korte, A. (1980) Chromosomal analysis in bone marrow cells of Chinese hamsters after treatment with mycotoxins. Mutation Res. 78:41-49.

Kram, D. and Schneider, E.L. (1978) Reduced frequencies of mitomycin-C induced sister chromatid exchanges in AKR mice. Hum. Genet. 41:45-51.

Kram, D., Bynum, G.D., Dean, R., Schneider, E.L., Farland, W.H., Williams, J.R. (1981) Effects of acute and chronic administration of mitomycin-C on the induction of sister chromatid exchanges in vivo. Environmental Mutagenesis 3:489-495.

Kram, D., Schneider, E.L., Senula, G.C. and Nakerishi, Y. (1979) Spontaneous and mitomycin-C induced sister chromatid exchange: comparison of in vivo and in vitro systems. Mutation Res. 60(3):339-348.

Kurvink, K., Bloomfield, C.D. and Cercenka, J. (1978) Sister chromatid exchange in patients with viral disease. Exp. Cell. Res. 113:450-453.

Lang, R. and Adler, I.D. (1977) Heritable translocation test and dominant lethal assay in male mice with methyl methanesulfonate. Mutation Res. 48:75-88.

Latt, S.A. (1973) Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. Proc. Nat. Acad. Sci. USA 70:3395-3399.

Latt, S.A. (1974a) Localization of sister chromatid exchanges in human chromosomes. Science 185:74-76.

Latt, S.A. (1974b) Sister chromatid exchanges, indices of human chromosome damage and repair: detection by fluorescence and induction of mitomycin-C. Proc. Nat. Acad. Sci. USA 71:3162-3166.

Latt, S.A. (1979) Sister chromatid exchanges. Genetics 92:83-95.

Latt, S.A. (1981) Sister chromatid exchange formation. Ann. Rev. of Genetics 15:11-55.

Latt, S.A. and Schreck, R.R. (1980) Sister chromatid exchange analysis. Am. J. Hum. Genet. 32:297-313.

Latt, S.A., Stetter, G., Juergens, L.A., Buchanon, G.R., Gerald, P.S. (1975) Induction by alkylating agents of sister chromatid exchanges and chromatid breaks in Fanconi's anemia. Proc. Natl. Acad. Sci. USA 72:4066.

Lederberg, J. (1981) Comparative toxicology, environmental health and national productivity. Am. J. Med. 70:9-11.

Lin, M.S. and Alfi, O.S. (1976) Detection of sister chromatid exchanges by 4'-6-diamindino-2-phenyl lindal fluorescency. Chromosoma 57:219-225.

Maier, P. and Schmid, W. (1976) Ten model mutagens evaluated by the micronucleus test. Mut. Res. 40:325-338.

Malling, H.V. and Valcovi, L.R. (1977) A biochemical specific locus mutation system in mice. Arch. Toxicol. 38:45-51.

Maltoni, C. and Lefemire, G. (1974) Carcinogenecity bioassays of vinyl chloride research plan and early results. Environ. Res. 7(3)387-405.

Maretoja, T., and Vainio, H. (1979) The use of human lymphocyte tests in the evaluation of potential mutagenesis: clastogenic activity of styrene in occupational exposure. Genetic Damage in Man Caused by Environmental Agents. edited by K. Berg, Academic Press, New York, pp. 213-225.

Marquardt, H. and Bayer, U. (1977) The induction in vivo of sister chromatid exchange in the bone marrow of the Chinese hamster. Mutation Res. 56:169-176.

Matter, B.E. and Grauweiler, J. (1974) Micronuclei in mouse bone marrow cells: A simple in vivo model for the evaluation of drug induced chromosomal aberrations Mutation Res. 23:239-249.

Merz, T. (1961) Effects of mitomycin-C on lateral root-tip chromosomes of Vicia faba. Science 133:329-330.

Natarajan, A.T., Csukas, I., and Van Zeeland, A.A. (1981) Contribution of incorporated 5-bromodeoxyuridine in DNA to the frequencies of sister chromatid exchanges induced by inhibitors of poly(ADP-ribose)-polymerase. Mut. Res. 84:125-132.

Natarajan, A.T., Tates, A.D., Van Buul, P.P.W., Meijers, M., and de Vogel, N. (1976) Cytogenetic effects of mutagens carcinogens after activation in a microsomal system in vitro. I. Induction of chromatid aberrations and sister chromatid exchange by diethylnitrosamine (DEN) and dimethylnitrosamine (DMN) in CHO cells in the presence of rat liver microsomes. Mut. Res. 37:83.

Nichols, W.W., Bratt, C.I., Toji, L.H., Godley, M. and Segawa, M. (1978) Induction of sister chromatid exchanges by transformation with simian virus 40. Canc. Res. 38:960-964.

Nowell, P.C. (1964) Mitotic inhibitions and chromosome damage by mitomycin in human leukocyte cultures. Exp. Cell Res. 33:445-449.

Painter, R.B. (1980) A replication model for sister chromatid exchange. Mutation Res. 70:337-341.

Pallitti, F., Tanzarella, C., Cozzi, R., Ricardy, R., Vitaliano, E., and Fiore, M. (1982) Comparison of the frequencies of SCEs induced by chemical mutagens in bone marrow, spleen and spermatogonial cells of mice. Mut. Res. 103:191-195.

Parry, J. (1972) Mitotic recombination in yeast as a test of genetic change. Laboratory Practice 21:417.

Pegg, A.E. (1977) Formation and metabolism of alkylated nucleosides: possible role in carcinogenesis by nitroso compounds and alkylating agents. Acad. Canc. Res. 25: 195-269.

Perra, F. and Mattias, P. (1976) Labeling of DNA and differential sister chromatid staining after BRdU treatment in vivo. Chromosoma 57:13-18.

Perry, P.E. and Evans, H.J. (1975) Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange, Nature (London) 258:121-124.

Perry, P.E. and Wolff, S. (1974) New giemsa method for differential staining of sister chromatids. Nature (london) 261:156-158.

Petras, M.L. and Topping, J.C. (1981) Studies of natural populations of Mus. VI. Sizes of populations inhabiting corn cribs in southwestern Ontario. J. Mammal. 62:146-153.

Popescu, N.C., Turnbull, D. and DiPaolo, J.A. (1977) Sister chromatid exchanges/chromosomal aberration analysis with the use of several carcinogens and non-carcinogens. J. Nat. Inst. 59:289-293.

Purchase, L.F.H., Longstaff, E., Ashby, J., Styles, D., Anderson, P.A.m Lefevre, P.A., and Westwood, F.R. (1976) Evaluation of six short-term tests for detecting organic chemicals, carcinogens and recommendations for their use. Nature (London) 264:624-627.

Ramirez, P.M. (1980) Analysis of in vivo sister chromatid exchange in mouse bone marrow and salivary gland cells. Mutation Res. 74:61-69.

Raposa, T. (1978) Sister chromatid exchange studies for monitoring DNA damage and repair capacity after cytostatics in vitro and in lymphocytes of leukaemic patients under cytostatic therapy. Mutation Res. 57:241-251.

Rao, T.K., Young, J.A., Weeks, C.E., Slaga, T.J. and Epler, J.L. (1979) Effect of co-carcinogens benzo(e)pyrene on microsome-mediated chemical mutagenesis in Salmonella typhimurium.

Renner, H.W. (1979) Monitoring of genetic environmental risk with new mutagenicity tests. Ecotoxicol. and Environ. Safety 3:122-125.

Roszinsky-Kocher, G. and Rohrborn, G. (1979) Effect of various cyclophosphamide concentrations in vivo on sister chromatid exchanges (SCE) and chromosomal aberrations of Chinese hamster bone marrow cells: Comparison of two methods measuring the mutagenicity of a test compound. Human Genetics 46:51-551.

Roszinsky-Kocher, G., Basler, A., and Rohrborn, G. (1979) Mutagenicity of polycyclic hydrocarbons: Induction of sister chromatid exchanges in vivo. Mutation Res. 66:

Rudiger, H.W., Kohl, F., Mangeles, W., Vonwichert, P., Bartram, C.R., Wohler, W. and Passage, E. (1976) Benzopyrene induced sister chromatid exchanges in cultured human lymphocytes. Nature (london) 262:290-292.

Russell, L.B. (1979) Meiotic non-dysjunction in the mouse: Methodology for genetic testing and comparison with other methods. Environ. Health Prospect. 31:113-122.

Russell, L.B. and Major, M.H. (1957) Radiation induced presumed somatic mutations in the house mouse. Genetics 42:161-175.

Russell, L.B. and Matter, B.E. (1980) Whole animal mutagenecity tests: Evaluation of five methods. Mutation Res. 75:279-302.

Russell, W.L. (1951) X-ray induced mutations in mice. Cold Spring Harbor Symp. Quant. Biology. 16:327-330.

Russell, W.L. (1977) The role of mammals in the future of chemical mutagenesis research. Arch. Toxicol. 38: 141-147.

Russev, G.C. and Tsanav, R.G. (1973) Continuous labeling of mammalian DNA in vivo. Anal. Biochem. 54:115-119.

Santi, D.V., McHenry, C.S. and Summer, H. (1974) Mechanism of interaction of thymidylate synthetase with 5-fluoro-deoxyuridylate. Biochem. 13:471-481.

Savage, J.K.R. (1976) Classification and relationship of induced chromosomal structural changes. J. Med. Gen. 12:103-122.

Schmid, W. (1975) The micronucleus test. Mutation Res. 31:9-15.

Schmid, W. (1976a) The micronucleus test for cytogenetic analysis. Chemical Mutagenesis 4:31-53, edited by A. Hollander, N.Y. Plenum Press, 1976.

Schmid, W. (1976b) Mutagen-carcinogen induced chromosome damage in human and mammalian cells in vivo and in vitro. Excerpta medica International Congress series No. 411, Proc. of the Fifth International Congress of Human Genetics, pp. 53-63.

Schneider, E.L. and Gilman, B. (1979) Sister chromatid exchanges and aging. III. The effects of donor age on mutagen induced sister chromatid exchange in human diploid fibroblast. Hum. Genet. 46(I):57-67.

Schneider, E.L., Sternberg, H and Tice, R.R. (1977) In vivo analysis of cellular repliaction. Proc. Natl. Acad. Sci. (USA) 74:2041-2044.

Schreck, R.R. (1980) Comparison of benzo(a)pyrene metabolism and sister chromatid exchange induction in mice. Nature 288:(5789):407-408.

Schreck, R.R., Paika, I.V. and Latt, S.A. (1979) In vivo induction of SCE in liver and marrow cells by drugs requiring metabolic activation. Mutation Res. 64:315-328.

Schubert, I. and Riger, R. (1981) Sister chromatid exchanges and heterochromatin. Hum. Genetics 57:119-130.

Schweiger, K.H. (1972) Untersuchung zur Strahlensensibilerierung der Induktion der Tryptophan Pyrrolase und der Tyrosin-Aminotransferase in der Leber der Man Dissertation Freibura, 1972.

Shiraishi, Y. (1978) Chromosome aberrations induced by monomeric acrylamide in bone marrow and germ cells of mice. Mutation Res. 57:313-327.

Shiraishi, Y. and Sandberg, A.A. (1978) Effects of mitomycin-C on normal and Bloom's Syndrome cells. Mutation Res. 49:239-248.

Singer, B. (1979) N-nitroso alkylating agents: Formations and persistance of aklyl derivatives in mammalian nucleic acids as contributing factors in carcinogenesis. J. Nat. Canc. Inst. 62:1329-1339.

Solomon, E. and Bobrow, M. (1975) Sister chromatid exchanges: A sensitive assay of agents damaging human chromosomes. Mutation Res. 30:273-278.

Speit, G. (1980) Effects of temperature on sister chromatid exchange. Hum. Genet. 55(3):333.

Stetka, D.G. and Wolff, S. (1976) Sister chromatid exchanges as an assay for genetic damage induced by mutagenic carcinogens. II. In vitro test for compounds requiring metabolic activation. Mutation Res. 41:343-350.

Stetka, D.G., Minkler, J. and Carrano, A.V. (1978) Induction of long-lived chromosome damage as manifested by sister chromatid exchange in lymphocytes of animals exposed to mitomycin-C. Mutation Res. 51:383-396.

Stetka, D.G., Minkler, J., Carrano, A.V., and Piluso, D.L. (1977) Sister chromatid exchanges induced by repeated exposure to mutagens carcinogens in vivo. J. Cell. Biol. 75:132.

Swenson, D.H., Harback, P.R. and Trzos, R.J. (1980) The relationship between alkylation of specific DNA bases and induction of sister chromatid exchanges. Carcinogenesis 1(11)931.

Szybalski, W. (1958) Special microbiological systems. II. Observations on chemical mutagenesis in microorganisms. Ann. N.Y. Acad. Sci. 76:475-489.

Takehisa, S. and Wolff, S. (1977) Induction of sister chromatid exchanges in Chinese hamster cells by carcinogenic mutagens requiring metabolic activation. Mutation Res. 45:263-270.

Takehisa, S. and Wolff, S. (1978) The induction of sister chromatid exchanges in Chinese hamster ovary cells by prolonged exposure to 2-acetylaminofluorene and S-9 mix. Mutation Res. 58:103-106.

Taylor, J.H. (1958) Sister chromatid exchanges in tritium labelled chromosomes. Genetics 43:515-529.

Taylor, J.H., Woods, P.S. and Hughes, W.L. (1957) The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium labelled thymidine. Proc. Nat. Acad. Sci. (USA) 43:122-128.

Tice, R.R., Costa, D.L. and Drew, R.T. (1980) Cytogenetic effects of inhaled benzene in murine bone marrow: Induction of sister chromatid exchanges, chromosomal aberrations and cellular proliferation inhibition in DBA/2 mice. Proc. Nat. Acad. Sci. USA 77:2148-2152.

Tsuchimoto, T. and Matter, B.E. (1979) In vivo cytogenetic screening methods for mutagens, with special reference to micronucleus test. Arch. Toxicol. 42:239-248.

Tsuda, H., Kushi, A., Yoshida, F. and Goto, F. (1981) Chromosomal aberrations and sister chromatid exchanges induced by gaseous nitrogen dioxide in cultured chinese hamster cells. Mutation Res. 89:303-309.

Ueda N., Eunaka, H. Akematsu, T., and Sugiyama, T. (1976) Parallel distribution of sister chromatid exchanges and chromosomal aberrations. Nature (London) 262:581-583.

Vig, B.K. (1977) Genetic toxicology of mitomycin-C, actinomycin, daunomycin and adriamycin. Mutation Res. 49: 189-238.

Vogel, W. and Bauknecht, T. (1976) Differential chromatid staining by in vivo treatment as a mutagenecity test system. Nature (London) 260:448-449.

Watanabe, M., Sachiko, H., Mikiko, H, and Takeshi, M. (1982) Mutagenic effects of combinations of chemical carcinogens and environmental pollutants in mice as shown by the micronucleus test. Mutation Res. 97(1)43-48.

Watanabe, M., Tamura, Y., and Abe, T. (1980) Sex-dependent differences in aryl hydrocarbon hydroxylase activity in mouse liver. Toxicol. Lett. 5:55-60.

Weisberger, J.H. (1976) Environmental cancer. J. Occup. Med. 18:245-252.

Wild, D. (1978) Cytogenetic effects in the mouse of 17 chemical mutagens and carcinogens evaluated by the micronucleus test. Mutation Res. 56:319-327.

Wolff, S. (1977) Sister chromatid exchanges. Ann. Rev. Genet. 11:183-201.

Wolff, S. (1978) Relations between DNA repair chromosome aberrations and sister chromatid exchange. In DNA Repair Mechanism, edited by P.C. Hanawalt, E.C. Friedberg and C.F. Fox. N.Y. Academic Press, pp. 751-760.

Wolff, S. (1981a) Induced chromosome variation In Chromosomes Today, Vol. 7, edited by M.D. Bennett, M. Bobrow and G.M. Hewitt, London, George Allen and Unwin, pp. 226-341.

Wolff, S. (1981b) Measurement of sister chromatid exchange in mammalian cells In DNA repair: A laboratory manual of research procedures, Vol. 1 part B, E.C. Friedberg and P.C. Hanawalt, editors, Dekker, N.Y.

Wolff, S. and Bodycote, D.J. (1975) The induction of chromatid deletion in accord with the breakage and reunion hypothesis. Mutation Res. 29:85-91.

Wolff, S. and Takehisa, S. (1977) Progress in Genetic Toxicology, edited by D.A. Scott, B.A. Bridges, and F.H. Sobels, Amsterdam, Elsevier, North Holland Biomedical Press, pp. 193-200.

Wolff, S., Bodycote, J. and Painter, R.B. (1974) Sister chromatid exchanges induced in Chinese hamster cells by u.v. irradiation at different stages of the cell cycle:

The necessity of cells to pass through S. Mutation Res. 25:73-81.

Wolff, S., Rodin, B., and Cleaver, J.E. (1977) Sister chromatid exchanges induced by mutagenic carcinogens in normal and xeroderma pigmentosum cells. Nature (London) 265:347-349.

Wynder, E.L., Gori, G.B. (1977) Contribution of the environment to cancer incidence: An epidemiologic exercise. J. Nat. Can. Inst. 58:825-832.

Yamamoto, K.I. and Kikuchi, Y. (1981) Studies on micronuclei, time response on the effects of multiple treatments of mutagens on the induction of micronuclei. Mutation Res. 90:163-173.

Zakharov, A.F. and Egolina, N.A. (1972) Differential spiralization along mammalian mitotic chromosomes. I. BUdR revealed differentiates in Chinese hamster chromosomes. Chromosoma 38:341-355.

Zimmerman, F.K. (1971) Induction of mitotic gene conversion by mutagens. Mutation Res. 11:327.

Zimmerman, F.K. (1975) Procedures used in the induction of mitotic recombination in the yeast Saccharomyces cervisiae. Mutation Res. 31:71.

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## PUBLICATIONS


1. Nayak, B.N. and Friars, G.W. (1968) A Selection Experiment Involving Genotype and Environment Interaction in Tribolium castaneum. Can. J. Genetics and Cytology 10:768 (Abstract)

2. Nayak, B.N. and Friars, G.W. (1969) Genotype and Environment Interaction in Tribolium castaneum Tribolium Inform. Bull. 11:85-86.

3. Nayak, B.N. (1969) Genotype and Environment Interaction in Tribolium castaneum. M.Sc. thesis, University of Guelph, Guelph, Ontario.

4. Friars, G.W., Nayak, B.N., Jui, P.Y., and Raktoe, B.L. (1971) An Investigation of Genotype and Environment Interaction in Relation to a Selection Experiment in Tribolium castaneum. Can. J. Genetics and Cytology 13: 144-154.

5. Friars, G.W., Nayak, B.N. and Jui, P.Y. (1971) Time Trends in Realized Genetic Correlation Between Traits Under Selection. Can. J. Genetics and Cytology 13: 623.

6. Nayak, B.N. (1972) The Application of Fluorescence Techniques to Sex Chromosome Determinations. M.Sc. (Anatomy) thesis, University of Western Ontario, London Ontario.

7. Friars, G.W., Nayak, B.N. and Hurnik, J.F. (1973) Realized Genetic Parameters Following Selection in Tribolium castaneum in Two Environments. Ann. Genetics Sel. Anim. 5:135-142.

8. Fairfull, R.W., Friars, G.W. and Nayak, B.N. (1973) Sire, Dam and Age Effects on Offspring Number in Tribolium castaneum. Triboleum Information Bulletin 17:91-93.

9. Nayak, B.N. and Petras, M.L. (1982) In vivo Sister Chromatid Exchanges and Genotoxicity Monitoring. An abstract presented at the Canadian Society of Genetics and Cytology, Calgary, Alberta.